HYGIENA STUDY :

AUDIT OF WOMEN MANAGED WITH CONE BIOPSY AT GROOTE SCHUUR HOSPITAL FROM 1ST APRIL 2010 TO 31ST OCTOBER 2013

Dr. Khatija Kadwa
KDWKHA001
MBCHB, Dip Obst (SA)
Department of Obstetrics and Gynaecology

Masters of Medicine

University of Cape Town

TABLE OF CONTENTS :

DECLARATION :

1. I know that plagiarism is wrong. Plagiarism is to use another's work and pretend that it is one's own.
2. I have used the ....HARVARD..... convention for citation and referencing. Each contribution to,
and quotation in, this essay/report/project/.....MMED..... from the work(s) of other people has been
attributed, and has been cited and referenced.
3. This essay/report/project/.....MMED..... is my own work.
4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his
or her own work.
5. I acknowledge that copying someone else's assignment or essay, or part of it, is wrong, and declare that this is my own work.

Signed:

Signed by candidate

Signature removed

K.Kadwa

LIST OF TABLES

LIST OF FIGURES:

ABSTRACT:

INTRODUCTION: Cervical cancer is the second commonest cancer in South Africa and the commonest amongst Black females with a Lifetime Risk (LR) of 1:35. In South Africa the problem has been compounded by the HIV epidemic as well as a lack of resources and infrastructure to offer an adequate screening and treatment programme. Cone biopsies are one of the diagnostic and sometimes therapeutic modalities used to assess and treat cervical precursors and cervical cancer. Unfortunately, cone biopsy of the cervix remains a morbid procedure often performed on young women in the reproductive age group and has resultant complications.

OBJECTIVE: To audit the demographics, indications, histology and post cone management and outcome of women requiring cone biopsies of the cervix, at Groote Schuur Hospital Colposcopy Clinic between 1st April 2010 and 31st October 2013.

METHODS: A group of women attending the colposcopy clinic, and requiring cone biopsies between 1st April 2010 and 31st October 2013 were identified from a computerized database, known as the Hygiena Database. Women who had an incomplete dataset were excluded. Folder review and review of the National Health Laboratory Services was also conducted. Patient demographics, indications, cone histology and follow up at 4-6 months, 10-12 months and > 12 months were analysed. Age, parity, HIV

status, CD4 count, ARV status and cone margin involvement were included in the univariate and multivariate analysis to determine predictors of persistent disease

RESULTS: Three hundred and seventy six cone biopsies were performed during the study period, with a mean age of 42.3 years, mean parity of 2. The majority of women [56,7% (213/376)] were HIV positive. The final histology indicated that 65,2% (246/376) of the women had high-grade disease (CIN 2/3 or HSIL) and 12,5% (47/376) had microinvasion. Ectocervical margins were clear in 57,6% (212/368) of cases and endocervical margins were clear in 54,6% (201/368) of specimens. Fifty-one cancers were detected during the study period. In the multivariate analysis age 40-49yrs (RR 1.4, 95% CI 1.01-2.0: p=0,043), ectocervical margin involvement with CIN 2/3 (RR 1.8, 95% CI 1.1-3.0: p-0.017) and endocervical margin involvement with CIN 2/3 (RR 1.5, 95% CI 1.04-2.3; p=0,031) and microinvasion (RR 2.4, 95% CI 1.4-4.3; p=0.003) were all predictors of persistent disease.

CONCLUSION: The use of cone biopsy is a valid diagnostic and sometimes therapeutic procedure at Groote Schuur Hospital with significant detection of high grade disease and cervical cancer. Women aged 40-49 years and positive cone margins are strong predictors of persistent disease. Improved compliance and a reduction in positive margins are two areas that need to be addressed to improve the current treatment programme. Use of cone biopsy as surgical therapy for early stage cancer appears promising but needs further study.

ACKNOWLEDGEMENTS

SUPERVISOR: Prof. Lynette Denny

Chief Specialist and HOD Obstetrics and

Gynaecology, GSH and UCT

CO SUPERVISOR: Dr. Nomonde Mbatani

Head : Gynaecological Oncology Unit

Department of Obstetrics and Gynaecology, UCT

STATISTICIAN: Mr. Henri Carrera

RESEARCH ASSISTANT: Janine Nixon

KEYWORDS /ABBREVIATIONS:

HPV - Human Papillomavirus

CIN - Cervical Intraepithelial Neoplasia

CERVICAL CANCER

COLPOSCOPY

LLETZ - Large loop excision of transformation zone

LEEP – Loop electrosurgical excision procedure

CONE BIOPSY

HIV - Human Immunodeficiency Virus

LSIL - Low grade squamous intraepithelial lesion

HSIL - High grade squamous epithelial lesion

MI - Microinvasion

ULNS - Upper limit not seen

AGUS - Atypical Glandular Cells

LVSI - Lymphovascular space invasion

AIS - Adenocarcinoma in situ

CHAPTER 1 : INTRODUCTION AND LITERATURE REVIEW

Magnitude of the Problem

Cervical cancer is the 4th commonest cancer in women globally, after breast, lung and colorectal cancer, and the seventh most common overall. More than 85% of the global burden occurs in developing countries where it accounts for 12% of all female cancers (Globocan 2012).

According to worldwide estimates of cancer compiled by the International Agency for Research on Cancer (IARC), 528 000 new cases of cervical cancer were diagnosed globally in 2012. High-risk regions, with estimated age standardized rates (ASRs) over 30 per 100,000, include Eastern Africa (42.7), Melanesia (33.3), Southern (31.5) and Middle (30.6) Africa. Rates are lowest in Australia/New Zealand (5.5) and Western Asia (4.4). Cervical cancer remains the most common cancer in women in Eastern and Middle Africa (Globocan 2012).

There is a significant discrepancy in the incidence of and mortality from cervical cancer in different regions of the world. There were an estimated 266,000 deaths from cervical cancer worldwide in 2012, accounting for 7.5% of all female cancer deaths. Almost nine out of ten (87%) cervical cancer deaths occurred in less developed regions. Mortality varied 18-fold between the different regions of the world, with rates ranging from less than 2 per 100,000 in Western Asia, Western Europe and Australia/New Zealand to more than 20 per 100,000 in Melanesia (20.6), Middle (22.2) and Eastern (27.6) Africa (Globocan 2012)

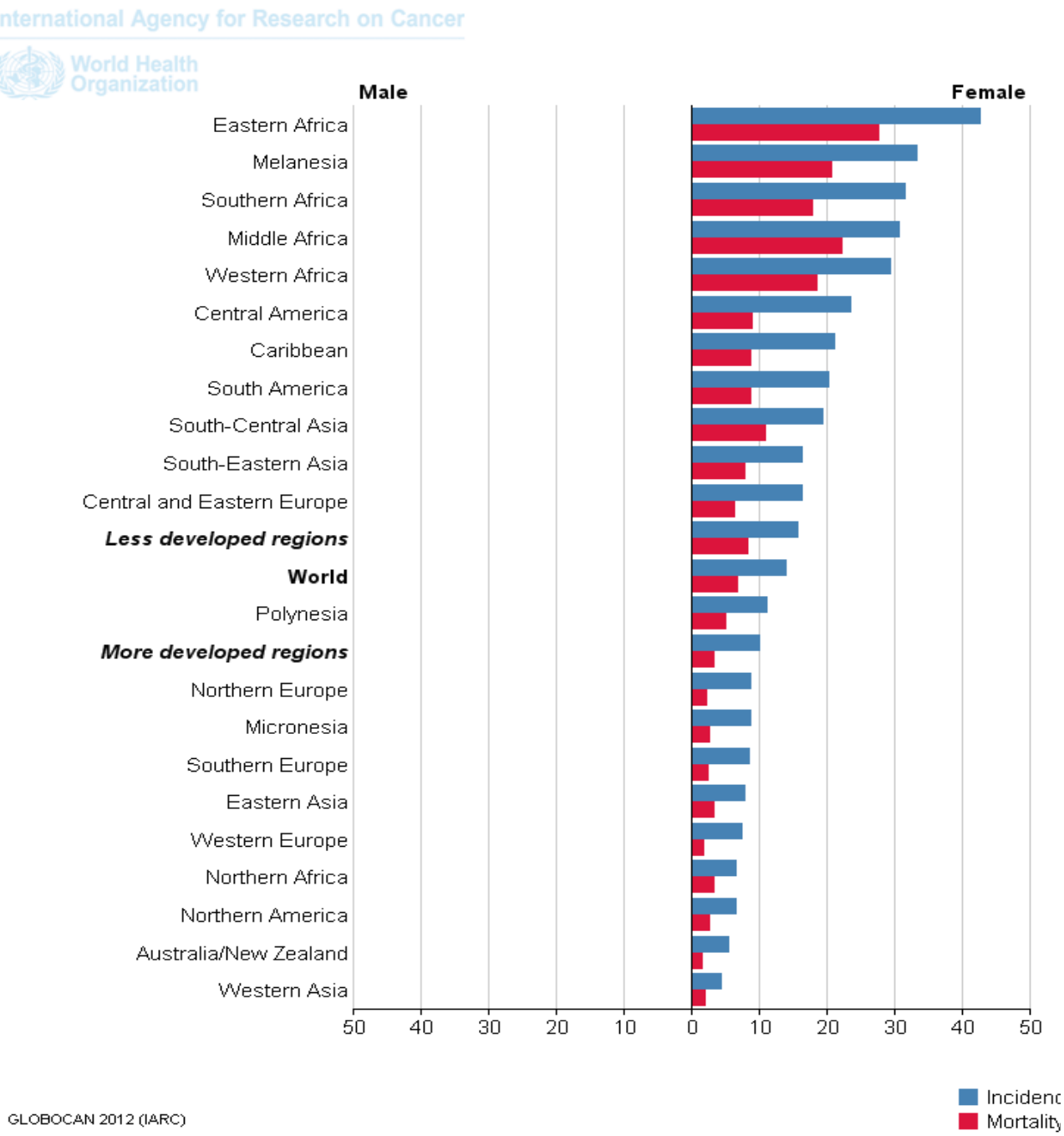


FIGURE 1.1 : Estimated Cervical Cancer Incidence and Mortality Worldwide in 2012 (International Agency for Research on Cancer, World Health Organization, 2012, GLOBOCAN 2012. Available from: <http://globocan.iarc.fr/.)

Cervical cancer in low resource settings

The marked disparity in cervical cancer incidence in low-versus high resource regions highlights the absence of effective national cervical cancer screening programmes in most low resource countries. A comprehensive analysis of data by the IARC in 1986 showed that well organised screening programmes were effective in reducing the incidence of and mortality from cervical cancer (IARC Working Group, 1986). In Scandinavian countries, national screening programmes implemented since the 1960's have significantly reduced cervical cancer incidence and mortality (Laara et al 1987; Hakama & Louhivuori 1988). The greatest fall in mortality was in Iceland (84% from 1965 to 1982) where the screening interval was the shortest and the target age range was the widest.

In the South African National Cancer Registry (pathologically based) in 2007, cervical cancer was the second most common cancer in women after breast cancer. It accounted for 18,1 % of all cancers in women, with ASIR of 21.78/100 000 and a lifetime risk (LR) of 1:42 for all South African women. The LR increased to 1:35 for Black South African women making it the most common cancer in this group, and accounting for 30% of cancers in black females. These statistics should be seen in the context of cervical cancer as a preventable and curable disease (National Cancer Registry Report 2007).

In Sub-Saharan Africa and South Africa in particular, the problem has been compounded by the HIV/Aids epidemic, where 70% of the world's cases are diagnosed [www.unaids.org]. According to Stats SA, the total number of persons living with HIV

in South Africa increased from an estimated 4 million in 2002 to 5,26 million by 2013. In 2013 an estimated 10% of the total population was calculated to be HIV positive (Statistics South Africa 2013). The prevalence of HIV among South African women attending antenatal clinics in 2011 was 29,5% (South African National Department of Health 2011). It is well documented that HIV infection increases the risk of developing certain cancers, namely Kaposi's sarcoma, Non-Hodgkin's lymphoma and cervical cancer, all of which were classified as AIDS- defining diseases by the Centre for Disease Control (CDC) in 1993(MMWR Recommendation Report 1992). According to Denny et al (2008), a strong relationship exists between HIV and HPV. The natural history of cervical neoplasia is one of progression and regression, partially mediated by local cervical immunity and a complex interaction with a range of co-factors (Stanley 2006). In HIV-positive women with cervical intraepithelial neoplasia (CIN), both pro-inflammatory and anti-inflammatory pathways in the cervical mucosa were found to be suppressed, in comparison with the local mucosal environment of HIV-negative women (Kobayashi, Greenblatt & Anastos et al 2004).

Natural History of Cervical Cancer

The evolution of cervical cancer has been well studied in the last half of the 20th century. A wealth of evidence now exists that implicates persistent infection with high risk /oncogenic types of HPV as the main cause of cervical cancer (Walboomers et al 1999). High risk HPV types are identified in nearly all types of carcinomas of the cervix and the relative risk of cervical cancer associated with high risk HPV types is higher than the risk of lung cancer associated with smoking (Munoz et al 2003).

The human papillomavirus is a tightly coiled, circular, double stranded DNA with 8000 base pairs in their genome. It is an obligatory intranuclear virus that must infect mitotically active cells to institute infection. HPV infection of the cervix affects the developing immature metaplastic cells of the transformation zone at the squamo-columnar junction, which explains why HPV is associated with both squamous and glandular neoplasia. Over 150 different HPV types have been identified, of which approximately 30-40% infect the anogenital tract. They are commonly classified into 15 oncogenic (HPV 16,18,31,35,39,45,51,52,56,58,59,68,73,82) and 12 non-oncogenic types (HPV 6,11,40,42,43,44, 54,61,70,72,81 and CP6108) (Munoz et al 2003). Pre malignant and malignant cells arise as result of DNA integration into the host cellular genome, and over expression of the viral E6 and E7 oncogenes. Cells acquire a proliferative advantage by escaping growth control exerted by p53 and pRb. Both cellular proteins are inactivated respectively by E6 and E7 proteins. Aneuploidy and karyotypic abnormalities are also key events in the tumour progression.

HPV is transmitted through sexual contact, and transmission occurs through mechanical abrasion of an infected epithelial surface with an uninfected epithelium. HPV's that are primarily transmitted through sexual contact, are found in 99% of cases of invasive cervical cancer. The incidence of HPV is highest amongst young women, and although most men and women can be infected during their sexual life, most are asymptomatic with transient infection. A small minority, with persistent infection will progress to dysplasia and subsequently to cancer (Cox 2006).

The vast majority of HPV viral infections are cleared within 8-18 months in women with competent immune systems. However, through a complicated cascade of events some

infections evade the immune system, becoming a persistent infection, which is necessary for the development of and progression of precancerous lesions of the cervix, either to high grades of precancerous disease or to cancer (Wright, Kurman & Ferenczy 2002). Cervical cancer precursors are now classified into low grade squamous intraepithelial lesions (LSIL)(previously cervical intraepithelial neoplasia 1) or high grade squamous intraepithelial lesions (HSIL) (CIN 2 and 3).

HPV and Cervical Precursor Lesions in HIV Infected Women

As previously mentioned, a strong relationship exists between HPV and HIV. HIV is thought to increase the risk of HPV replication or transcription by a direct viral-viral interaction (Dolei, Curreli & Marongei 1999; Vernon, Haart, Reeves & Icenogle 1993).

Studies have consistently shown higher prevalence of HPV infection, persistent infection with HPV, infection with multiple types of HPV and higher prevalence of cervical cancer precursors in HIV infected women and presentation at an earlier age with cervical cancer (Singh et al 2009; Ellerbrock et al 2000; Harris et al 2005).

In 2005, a prospective observational study of 710 HIV positive and 226 HIV negative women, was undertaken in Rwanda. The prevalence of HPV was higher in HIV positive women compared to HIV negative women, with a peak of 75% in the 25 – 34 year age group and then declined with increasing age to 37.5% in those over 55years ($p<0,001$). Among the HIV positive women, 35% were infected with multiple types of HPV and 46% with carcinogenic HPV types. A significant trend of higher prevalence of HPV and

carcinogenic HPV with lower CD4+cell counts and increasing cytologic severity was also noted amongst HIV positive women (Singh et al 2009).

These findings were further supported by a recent study by McDonald et al (2014) in Cape Town, South Africa where 1 371 HIV positive women and 8 050 HIV negative women with no history of cervical screening were recruited from the general population. All the women were tested for HIV, cervical samples were tested for high risk HPV DNA and CIN status was based on colposcopy and biopsy. They found a higher prevalence of HPV amongst HIV positive compared to HIV negative women (52,4% vs. 20,8%) overall and in all age groups. High grade lesions, infection with high risk HPV types and multiple HPV types was more common among HIV positive than HIV negative women.

HIV positive women have higher rates of HPV and cervical abnormalities than HIV negative women. The natural history of HPV infection and cervical disease in HIV-1 infected women was studied by Denny et al (2008) who found that 68% of their cohort of HIV positive women were infected with high risk types of HPV with 94% of these infections persisting over a 36 month period and only 6 % clearing their infections. Overall, 35% of women had a LSIL lesion on Pap smear and 13% HSIL on entry.

The vast majority of LSILs regress spontaneously in immunocompetent women, however HIV positive women with LSIL have a lower rate of regression and more tendency to progression. Massad et al (2001) noted progression of cervical dysplasia in 14% of HIV infected women compared to 7% of HIV negative women, with regression to normal being lower in HIV positive women (43% vs. 66%.). HIV status, HPV detection of high risk types, CD4 count and viral load predicted the incidence of cytologic abnormalities.

Several studies have shown that invasive cervical cancer in HIV positive women tends to present 10-15years earlier than their seronegative counterparts, and those with CD4 counts <200 present with more advanced stage disease (Lomalisa, Smith & Guidozzi 2000 ; Moodley, Moodley & Kleinschmidt 2001).

As demonstrated above women infected with HIV, have an increased risk of being infected with HPV, multiple strains of oncogenic HPV, high grade lesions and therefore cervical cancer. However, the expected increase in cervical diagnosis in Africa during the HIV pandemic has not been convincingly observed. This is most likely due to women dying of opportunistic infections prior to developing cervical cancer. However, in the era of anti-retrovirals where women are expected to live longer, the risk of high grade cervical lesions and cervical cancer remains high.

Cervical cancer progresses slowly over decades, from preinvasive cervical intraepithelial neoplasia (CIN) to invasive cervical cancer- a process that can take 10-30 years. The long natural history of the disease offers a unique opportunity for screening and prevention prior to the development of invasive cancer. In addition the long latency period between primary infection and cancer emergence suggests that additional factors are involved in the process such as, among others, sexual behavior, immune status, genetic predispositions, nutritional status, smoking and socio-economic level (Mougin et al 2001).

Cervical Cancer prevention and treatment

Cervical cancer prevention involves a two tiered approach. Primary prevention involving safe sexual practices and HPV vaccination and secondary prevention comprising cytology (pap smears), visual inspection of the cervix and HPV testing.

A vaccine against HPV has been one of the most exciting recent developments in cervical cancer prevention. The two vaccines currently available offer high levels of protection against persistent infection with oncogenic strains of HPV 16 and 18 responsible for 70% of cervical cancers worldwide. These vaccines are prophylactic and require administration before the onset of sexual activity i.e. in prepubertal females. Studies by Goldie at al (2004), using modeling, and assuming 70% coverage of girls 9-12yrs with vaccination against HPV 16 and 18, have shown a 43% reduction in lifetime risk of cervical cancer.

In addition HPV DNA testing has offered us another option in the arsenal of cervical cancer screening. Studies by Denny et al (2005 &2012) and Sanakaranarayanan et al (2007 & 2009), have shown that HPV DNA testing in combination with VIA (Visual inspection with acetic acid) or alone are effective in reducing high grade lesions, and the incidence of cervical cancer and deaths particularly if screening is linked with treatment. Visual inspection with acetic acid (VIA) , involves examination of the cervix after application of 3-5% acetic acid, using the naked eye aided by a bright light source.

Unfortunately, in most low resource settings such as found in Africa, the above interventions have remained prohibitively expensive, and cervical cancer screening has relied on the detection of cervical cancer precursors using cervical cytology via the

Papanicolaou (pap) smear. There are few, if any, successful cytology-based cervical cancer screening programmes in any developing countries, hence the large burden of disease.

The South African National cervical screening programme offers 3 free Pap smears for asymptomatic women, beginning at age 30 and each performed 10 years apart (South African National Department of Health 2003).

Referral criteria for colposcopy include :

- 3 consecutive smears with atypical squamous cell of undetermined significance (ASC-US) or 2 smears with low grade squamous intraepithelial lesion (LSIL)
- 1 smear showing ASC – H (atypical squamous cells – HSIL cannot be excluded
- high grade squamous intraepithelial lesion (HSIL)
- Atypical Glandular lesions
- macroscopically suspicious lesion of the cervix or Pap smear suspicious for microinvasion

For women known with HIV, the clinical guidelines recommend a Pap smear at HIV diagnosis and with follow up every 3 years if normal, regardless of antiretroviral therapy (ART) status. However, referral of HIV positive women for colposcopic evaluation is recommended after the first abnormal smear.

At Groote Schuur Hospital, if a high grade precursor lesion is visualized at colposcopy, one of two approaches is used. Either a biopsy is taken to confirm the diagnosis or an

immediate LLETZ is performed. The criteria for LLETZ (considered a therapeutic procedure) include :

- parity between cytology and colposcopic diagnosis
- upper limit of lesion is seen
- no evidence of glandular abnormality
- no evidence of microinvasion

By contrast, if the above criteria are not met a diagnostic cone biopsy is performed under the following circumstances :

- marked disparity between cytology and colposcopy e.g. HSIL pap but negative colposcopy
- upper limit of lesion not seen
- evidence of glandular abnormality
- evidence of microinvasion

However, where a desire for future fertility exists, confirmatory biopsies are required before an excision procedure, which may be a LLETZ or Cone Biopsy.

Cone biopsy of the cervix involves excision of the transformation zone and the endocervical canal as a cone shaped specimen, and is considered a diagnostic procedure, however it can be therapeutic. It may be performed via loop diathermy (hot loop/electrodiathermy) or with a surgical scalpel (cold knife) and remains distinctly separate from LLETZ, which is a therapeutic procedure.

Unfortunately, cone biopsy of the cervix remains a morbid procedure often performed on young women in the reproductive age group and has resultant complications. Early complications include primary and secondary haemorrhage, sepsis and cervical stenosis and cervical incompetence. Late complications range from dysmenorrhoea, cervical stenosis resulting in infertility (sometimes requiring assisted reproductive techniques) and pregnancies associated with preterm prelabour rupture of membranes, preterm labour and low birth weight infants (Sozen et al 2014; Sadler et al 2004; Fanfani et al 2014). Sadler et al (2014) compared the delivery outcome of 426 untreated women with 652 treated women (via cone biopsy, laser ablation or LLETZ) at a colposcopy clinic in Auckland, New Zealand. The overall rate of preterm delivery was 13,8% and LLETZ and cone procedures were associated with a significantly increased risk of preterm rupture of membranes. This risk increased with increasing height of tissue removed from the cervix at conization. These findings were confirmed in a study by Sozen et al (2014) who found a significantly higher rate of preterm delivery and preterm prelabour rupture of membranes in the cohort of patients previously treated with cone biopsies. Cervical tissue volume rather than height of excised cervical tissue was found to be a more significant factor.

Cone biopsies have also have an increasing role in oncology surgery for early stage cervical cancer. Particularly in the arena of fertility sparing surgery, it offers an alternative to hysterectomy for patients with stage early stage squamous carcinoma and adenocarcarcinoma in situ. Less radical surgery in the form of cone biopsy (with

adequate margins) and sentinel nodes has offered another option to hysterectomy, thus alleviating the morbidity associated with more radical surgery (Lee et al 2014). Bouchard Fortier, Reade & Covens (2014) compared the outcomes of women with early stage cancer (stage 1Ai to 1Bi) treated with cone biopsy and pelvic lymphadenectomy versus simple hysterectomy and lymphadenectomy. Twenty two (43%) and twenty nine (57%) women underwent simple hysterectomy and cone biopsy respectively. The median tumour size was 10mm, median depth of invasion 2mm and lymphovascular space invasion (LVSI) was present in 35% of women. Surgical margins were all negative. Two women received adjuvant chemoradiaton for deep stromal invasion with LVSI and the other for pelvic metastases. Of the remainder, none had post- or intraoperative complications, the median blood loss was 100ml and none of the 51 women developed a recurrence during follow up. The follow up was a median of 21 months with a range of 1-112 months. They concluded that non-radical surgery resulted in low complication rate and excellent surgical out comes in well selected patients.

Fanfani et al (2014) looked at the sexual and reproductive outcomes in women with early stage cervical cancer after excisional cone biopsy and laparoscopic lymphadenectomy, as fertility sparing surgery, in Italy. Twenty three patients with an average age of 30 years participated in the study. After the treatment, all the women had regular menstruation with 30% reporting increased dysmenorrhea. One (4,4%) experienced cervical stenosis. Of the 10 patients that attempted conception, 6 (60%) conceived spontaneously whilst 4 (40%) underwent in vitro fertilization and embryo transfer with 1 (25%) being successful.

Sexual complaints included inadequate lubrication, performance anxiety and dyspareunia, which resolved in the subsequent 3 months.

Persistence and recurrence of Cervical disease

In addition to being a therapeutic and diagnostic procedure, certain aspects related to the cone biopsy may predict persistent or recurrent disease. Numerous studies have been performed looking at different variables with respect to demographics and cone histology as predictors of persistent or recurrent disease. Many of these have shown conflicting results. Moore et al in (1995) reviewed 1272 patients of whom 311 had a subsequent hysterectomy within one year. The initial histology was HSIL in 57.3% of cases (727/1272) and 3.2% showed invasive cancers (42/1271). Findings were either normal or low grade SIL in 40% (53/1272) cases. Residual disease was defined as CIN or cancer in the hysterectomy specimen. The following factors were evaluated for predictive value of residual disease : age, race, gravidity, parity, socioeconomic status, cigarette smoking, marital status, degree of dysplasia, margin and endocervical gland involvement and status of the endocervical curettage. They found that 34% (106/311) of hysterectomy specimens had residual disease and by multivariate analysis only increasing age and degree of dysplasia were predictive of residual disease. Margin involvement was not predictive in this study. The concern with this study is the high degree of persistent disease in the hysterectomy specimens which may suggest poor technique.

A recent study by Shaco-Levy et al (2014) also found older age ($p=0.03$) to be associated with persistent/recurrent disease as well as involvement of the cone margins (focal hazard ratio = 17, p,0.001; extensive : hazard ratio =28, $p< 0.001$).

In contrast, Ramchandi et al (2007), found age not to be a predictor of recurrence or residual disease. In their multivariate analysis, the endocervical curettage and the endocervical margin status were significant predictors of recurrent/persistent disease. However when the degree of dysplasia and the ectocervical margin status were included in the multivariate analysis, endocervical margin status (OR 6.761; 95% CI, 2.7-17.2) and severity of cervical disease (OR, 1.930; 95% CI 1.038,3.59) were the only statistically significant predictors of persistent/recurrent cervical neoplasia. Postmenopausal status was also shown to be a predictor in another study (Saeaib et al 2009). Many studies have found severe degree of dysplasia, multiple quadrant involvement and positive margins to be predictive of persistence/ recurrence (Saeaib et al 2009; Tillmanns et al 2006; Moore et al 1995; Ramchandani et al 2007; Leguevaque et al 2010; Lubrano et al 2012 & Shaco-Levy et al 2014).

HIV status is another aspect that has been studied in relation to persistence/recurrence of disease. Massad et al (2007) looked at the outcomes after treatment of cervical intraepithelial neoplasia among women with HIV. Women in two prospective cohort studies, the Women's Interagency HIV Study (WIHS) and the HIV Epidemiology Research Study (HERS) were followed up 6 monthly after treatment with CIN using HPV testing and cytology with colposcopy as indicated. Identification of CIN or SIL (squamous intraepithelial neoplasia) within 6 months was defined as treatment failure and later disease as recurrence. Follow up was available for 170 HIV positive and 15 HIV negative women. Treatment failed in 45% of women (79 HIV positive and 5 HIV negative).

Failure was noted to be more likely in women with

- lower CD4 counts(<200 cell/microL: OR =2.96, 95% CI 1.4-6.20)
- detectable HPV DNA (OR 8.2; 95% CI 1.8-37.4, p=0.01)

In the multivariate analysis of HIV positive women, recurrence was more likely among:

- women treated for CIN 2,3 (HR =2.4; 95% CI 1.4 - 4.8)
- those with CD4 counts of less than 200 cells/microL
- oncogenic HPV infection (HR 2.9 ; 95% CI 1.4-6.1)

Most failures and recurrences were low grade, with one adenocarcinoma diagnosed. They concluded that treatment failure and recurrence was more common in women with HIV but is usually low grade (Massad et al 2007) .

Babkina et al (2014) conducted a case control study to investigate the outcomes of cervical conization for CIN 2 and 3 in HIV positive women and age- matched HIV negative controls and to determine whether positive margins, positive endocervical curettage, CD4 count or viral load were associated with the persistence of CIN2,3 or residual CIN 2,3 on specimens from repeat excision procedures or hysterectomy. Persistent CIN 2/3 was diagnosed in 28 HIV positive women (63%) and 14 HIV negative women (31.8%). In HIV positive patients, a positive margin was associated with higher persistence after cone biopsy (OR 5.3, 95% CI 1.7-24.14) and 75% had residual CIN 2/3 on a repeat procedure compared to 45% of controls. In HIV negative patients, positive endocervical curettage was associated with a higher persistence rate. However CD4 count or viral load was not associated with residual or persistent. Unfortunately, this was a small study, with 44 HIV positive patients and 44 age matched controls.

Recent studies have also investigated and positively correlated disease persistence and recurrence with HR-HPV infection (Leguevaque et al 2014 & Lubrano et al 2014). Unfortunately testing for infection of the cervix for HPV is not offered at GSH at present.

In the context of a low resource setting with a high incidence of cervical cancer, further compounded by the HIV epidemic, it is imperative that we offer our patients a good quality screening and treatment program. One of these modalities, is the cone biopsy, being a diagnostic and sometimes therapeutic procedure but not without morbidity, especially for women in the reproductive age group. Cone biopsies, are also being used as a treatment option for early stage cancer.

Considering the morbidity associated with cone biopsy, this study aimed to audit the indications and outcome of women requiring cone biopsies of the cervix, at Groote Schuur Hospital Colposcopy Clinic between 1st April 2010 and 31st October 2013. In order to have a better understanding of the spectrum of disease we aimed to review patient demographics with respect to age, parity, contraception and HIV status. Where patients were HIV positive, CD4 count and ARV status were documented. Referral cytology, indications for cone biopsy, histological outcome and post cone management and follow up were reviewed. While studies have been conducted elsewhere (mostly in developed countries) regarding the use of cone biopsies, their outcomes, predictors of persistent disease and their use in early stage cancer, scanty information regarding the use of cone biopsies is available in our local setting. This study aimed to review current

practice and make recommendations for improvement of the service as well as the use of cone biopsies for early stage cancer.

CHAPTER 2 : METHODS

The Hygiena database was established in April 2010 at the Colposcopy Clinic, Groote Schuur Hospital. All patients attending the colposcopy clinic have their demographic details, medical history, clinical complaints and results captured on a structured proforma (Appendix A-D), which is entered into the Hygiena Database. Ethics approval was attained (HREC REF: 344/2011) on establishment of the database.

A group of women attending colposcopy clinic whose data was entered into our anonymised, computerized database and who required a cone biopsy between the 1st April 2010 and 31st October 2013 were identified. The data for these women were included in the analysis. This allowed for a 10 month follow up of patients until 31st July 2014. Women who did not receive a cone biopsy or had an incomplete dataset were excluded.

Women included in the study were those that required an excisional procedure, in this case a cone biopsy, either following a confirmatory biopsy of a high grade lesion or immediately after visualization of a high grade precursor at colposcopy. Women with marked disparity between cytology and colposcopy (e.g. HSIL pap but negative colposcopy), where the upper limit of the lesion was not seen, where there was evidence of a glandular abnormality or evidence of microinvasion, all had cone biopsies performed and were included in the study. Cone biopsy of the cervix involves excision of the transformation zone and the endocervical canal as a cone shaped specimen and is performed via loop diathermy (hot loop) in the colposcopy clinic or with a surgical

scalpel (cold knife) in main theatre. The vast majority of women in the study had cone biopsies performed as an outpatient procedure (hot loop cone).

All patients were identified by a study number linked to their hospital folder number to ensure confidentiality.

Data extracted from the database included:

- date of registration
- date of birth
- age
- parity
- contraception
- HIV status with CD4 count and ARV status if HIV positive.
- referral cytology or cytology prior to cone biopsy
- indication for cone biopsy

HIV status was documented as positive, negative or unknown. While the proforma documented CD4 counts as a range i.e. < 200, 200-350 etc. every attempt was made to document absolute CD4 counts by confirmation with National Health Laboratory Service (NHLS) and folder review. If the absolute CD4 count was unknown a median of the range documented was included for analysis.

Cytology that precipitated the cone biopsy either at referral or prior to cone biopsy being performed at subsequent visits, was included for analysis. Cytology was categorized into normal, recurrent LSIL, HSIL, adenocarcinoma in situ (AIS) or microinvasion.

There are four main indications for a cone biopsy and these include upper limit of lesion not seen (ULNS), disparity between cytology and colposcopy, microinvasion and atypical glandular cells (AGUS) on pap smear. The fifth, being absence of colposcopy, was not relevant to our study. Where the indication for the cone biopsy was unclear, review of the histology and clinical information provided as well as folder review was conducted to confirm the indication.

The histology of all the cone biopsies performed between 1st April 2010 and 31st October 2013 was reviewed on the NHLS database and categorized as normal, LSIL, HSIL, AIS or microinvasive cancer. Cone biopsy margins with respect to the ectocervical and endocervical margins were also documented. The denominators may vary in the analysis according to the availability of information regarding margins, which was missing in some cases.

Folder review as well as review of subsequent histology and cytology was conducted to assess post cone biopsy management and compliance with follow up. Visits post cone biopsy at 4-6 months, 10-12months and more than 12 months were documented, with respect to intervention/treatment and relevant cytology and histology at these visits. Patients were categorized as lost to follow up or the end point, i.e. the most invasive procedure required for treatment, was documented. . Hence patients were categorized as

lost to follow up, follow up only i.e. pap smear/cervical biopsy, simple hysterectomy or radical hysterectomy. Patients with invasive cancer requiring radiotherapy, or those deemed medically unsuitable for surgery and requiring radiotherapy, were excluded from this analysis.

Statistical analysis was performed using Stata version 13.1 (StataCorp LP,4905 Lakeway Drive, College Station, TX 77845, USA). The distribution of continuous data was assessed graphically and using the Shapiro Wilk test. Normally distributed data were summarised using means and standard deviations and analysed using parametric methods (independent or paired t-tests as appropriate, or analysis of variance for the comparison of 3 or more means). Skewed data were summarised using medians and interquartile ranges and analysed using non-parametric methods (Mann-Whitney-U-tests or Wilcoxon signed rank tests for paired data as appropriate, or the Kruskal Wallis test for the comparison of 3 or more medians).
Proportions were compared using Chi-squared tests or Fisher's exact test as appropriate.

Binomial 95% confidence intervals were used to report the precision of binary variables.

The relationship between binary outcome data and explanatory variables were analysed using prevalence ratios and 95% confidence intervals.

RESULTS :

Four hundred and twenty potential cone biopsies were identified on the Hygiena database, between 1st April 2010 and the 31st October 2013. Forty four patients were excluded due to missing data or the procedure being a LLETZ or punch or wedge biopsy. Three hundred and seventy six patients were included in the analysis.

Demographics

The mean age of the study sample was 42,3 years, with the youngest being 23 and the oldest 79 years of age. Parity was known for 375 women, with the majority of women 30,4% (114/375) having had 2 children, with a range of 0-11.

When considering women in the reproductive age group (i.e. 23-45years for our study), just over a third of these women 35,0% (85/243) were not using any form of contraception. For those who were, the most common was the long acting injectable used by 36,2% (88/243) of women, two of whom were additionally using condoms. Other methods were condoms at 16,4% (40/243) and sterilization 10,3% (25/243). A minority of women 2,0%(5/243) used the Combined Oral Contraceptive.

The HIV status was negative for 31,9% (120/376) of women, unknown in 11,4% (43/376) cases and positive in 56,7% (213/376) of women [95% CI51.6-61.6]. This is graphically presented in figure 2.1 below.

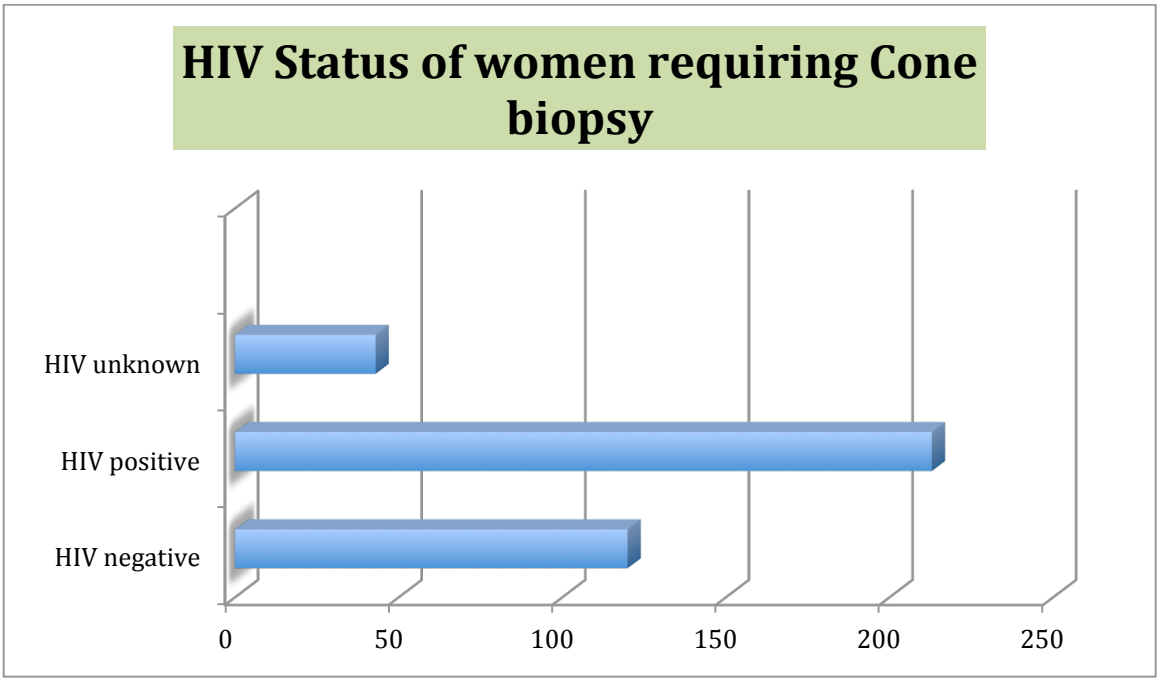


FIGURE 2.1 : HIV Status of women requiring Cone Biopsy

The CD4 count was known for 205 of the 213 HIV positive females, the median CD4 being 310, with the range from 5 to 1303. The majority of these women 32,7% (67/205) had CD4 counts between 200 and 349. The rest of the categories i.e. 0-199, 350-499 and CD4 > 500 all ranged between 22,0% to 22.9% as shown in Table 1 below.

In addition, 70,9% (151/213) of the HIV positive women were on antiretroviral treatment (ART), 26,3% (56/213) were not on treatment and ARV status was unknown for 8 women.

		CD4 0-199	CD4 200-349	CD4 350-500	CD4>500
CD4 known	205	22,4% (46/205)	32,7% (67/205)	22,0% (45/205)	22,9% (47/205)
CD4unknown	8				

TABLE 1. Distribution of CD4 counts amongst HIV positive patients

Histology and Cytology of Cone Biopsy

The indications for cone biopsy were as follows: 30% (112/376) for disparity between cytology and colposcopy [95% CI 26,1-43,6], 30,6% (115/376) for suspected microinvasive disease [95% CI 26,1-35,4] and 38,6% (145/376) for upper limit of the lesion not seen [95% CI 33,7-43,6]. Only 4 patients (1,1%) had suspicion for a glandular lesion. This is illustrated in figure 2.2

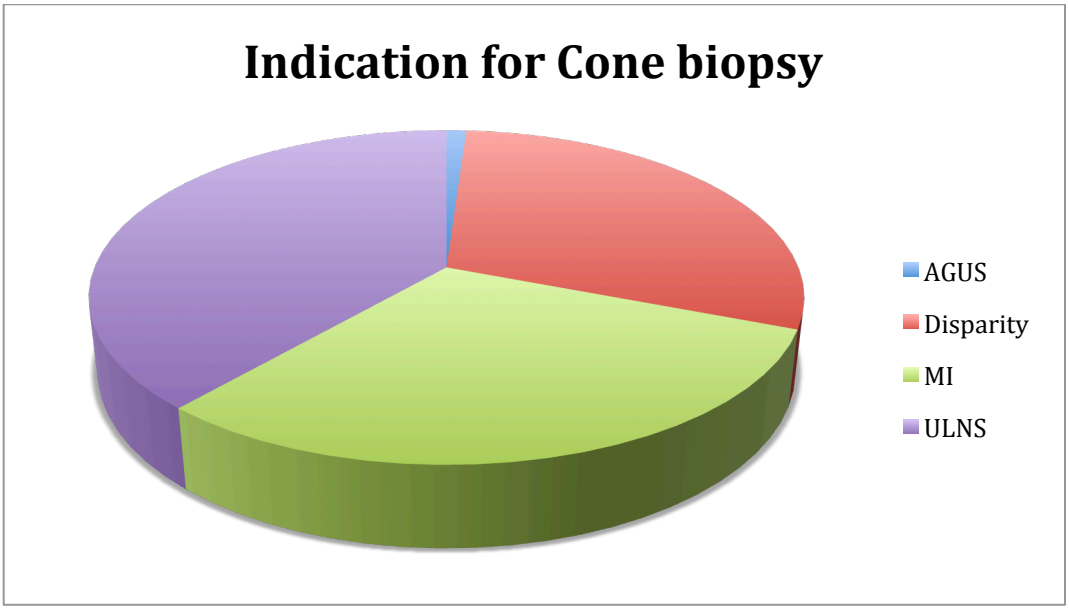


FIGURE 2.2 Indications for Cone Biopsy

The final histology of the cone biopsies indicated that the majority of patients i.e. 65,4% (246/376) had high-grade cervical cancer precursors (CIN 2,3)[95% CI 9.5-16.2]. Of the remainder, 14,4% (54/376) had CIN 1 [95% CI 11.2-18.3], 12,5% (47/376) had microinvasion [95% CI 9.5-16.2], 1,3% (5/376) had AIS [95% CI 0,5-3.2], 5,9% (22/376) had no disease [95% CI 3.9-8.7] and in 2 cases the specimen was suboptimal for evaluation. Hence the negative cone rate was 5,9%. These results are shown in Figure 2.3.

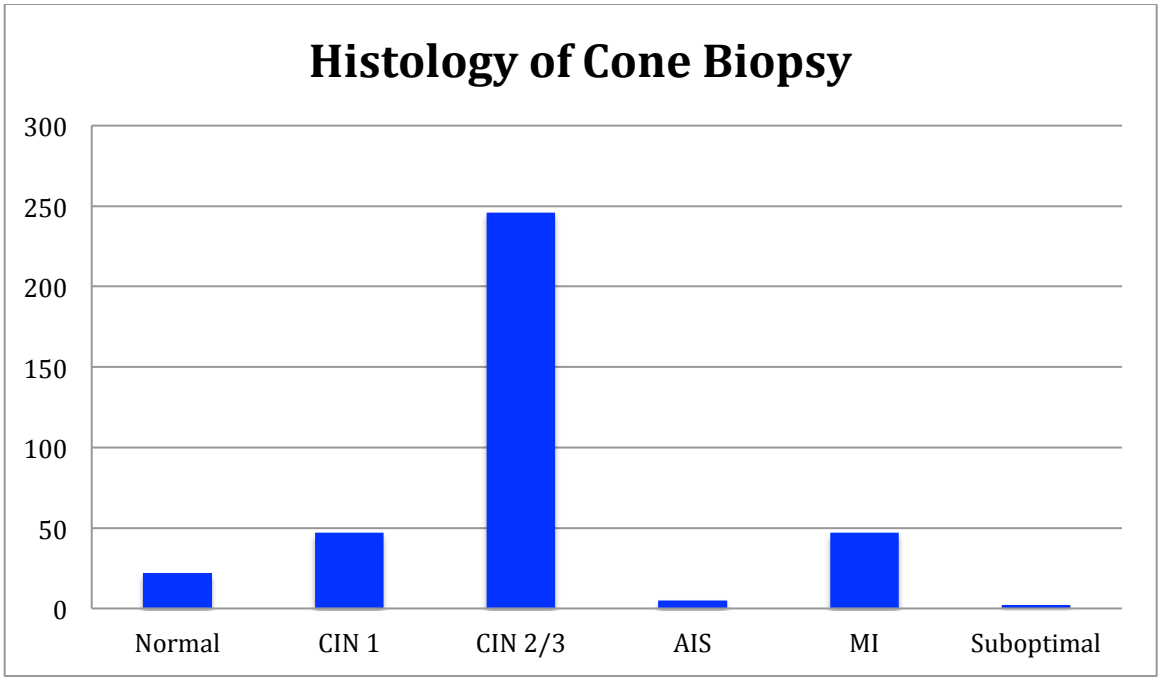


FIGURE 2.3 Histology of Cone Biopsies performed

Both the ecto- and endocervical margins were reported on in 96,6% (363/376) of specimens [95% CI 94.1-98.0]. There were cautery artifacts, lost epithelium or no report in 9 cases and only the endocervix was reported on in 4 cases.

Where the ectocervix was reported upon, it was clear in only 57,6% (212/368) of cases [95% CI 52.5-62.6]. But where involved, this was due to CIN1 in 18,8% (69/368) of specimens [95% CI 15.1-23.1] and CIN 2/3 in 18,5% (68/368) of cases [95% CI 14.8-22.8]. Microinvasive cancer was involved in 3,5% (13/368) of cases, AIS in 1 specimen and 4 were suboptimal.

Endocervical margins were only clear in 54,6% (201/368) of specimens. When involved, the majority of margins 33,2% (122/368) were involved with CIN2/3. In 6,3% (23/368) the endocervix was involved with microinvasive disease, 5,4% (20/368) with CIN 1 and 1 patient had AIS. One patient had no data (Figure 2.4).

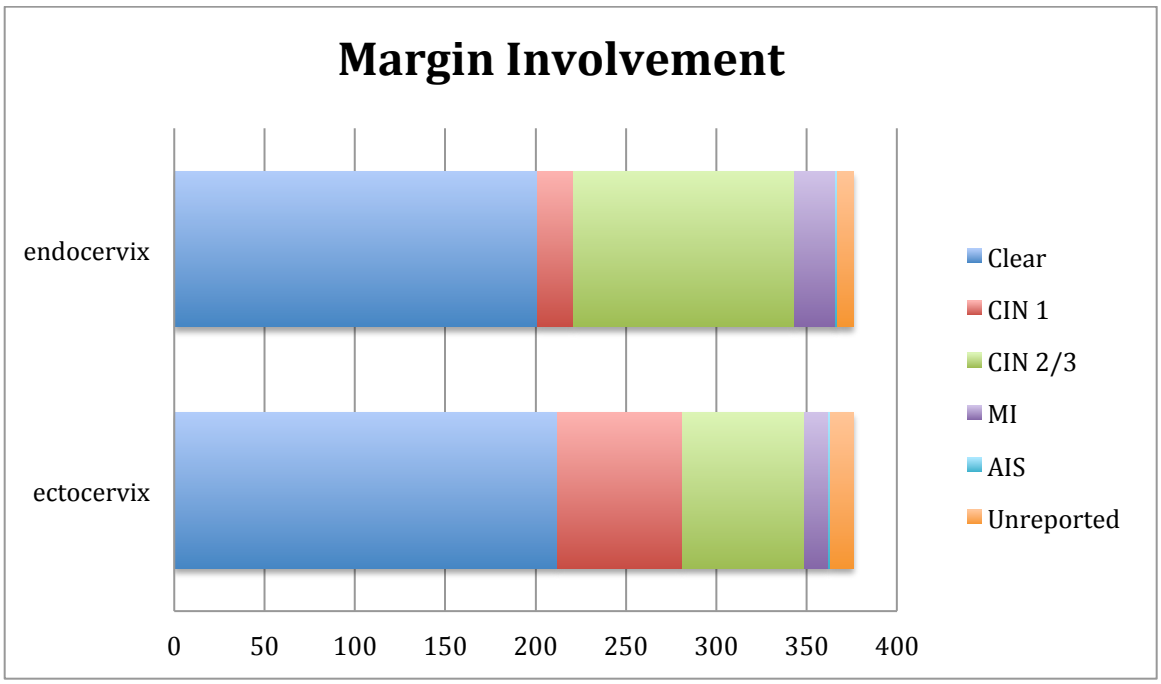


FIGURE 2.4 Ectocervical and Endocervical Cone Margin Involvement

Forty seven cases of microinvasion were detected via cone biopsy. Four cancers were detected on LLETZ prior to cone biopsy with the subsequent cone biopsies showing CIN 1 or CIN 2/3. The vast majority of the cancers i.e. 84,3 % (43/51) were squamous carcinoma, with 9,8% (5/51) being adenocarcinoma and 3 equally adenoid basal, adenoid cystic and adenosquamous carcinomas.

Of the 51 cancers detected 26 were stage 1A (56%), of which 21 were 1Ai. Sixteen of the 51 cancers (33,3%) were stage 1B1 and one was 1B2. Seven patients had stage 2 disease and 1 patient Stage 4 cancer.

Post cone management and follow up

When reviewing the number of visits post cone biopsy, it is apparent that follow up was relatively poor in this study group. Eleven patients were excluded from the analysis due to follow up with radiation oncology and one having demised. Of the 365 remaining patients, 17,3% (63/365) never returned, 31,8% (116/365) returned for 1 visit, 23,8% (87/365) for 2 visits, 28.8% (105/365) for 3 visits and only 5 patients had 4 visits (Figure 2.5)

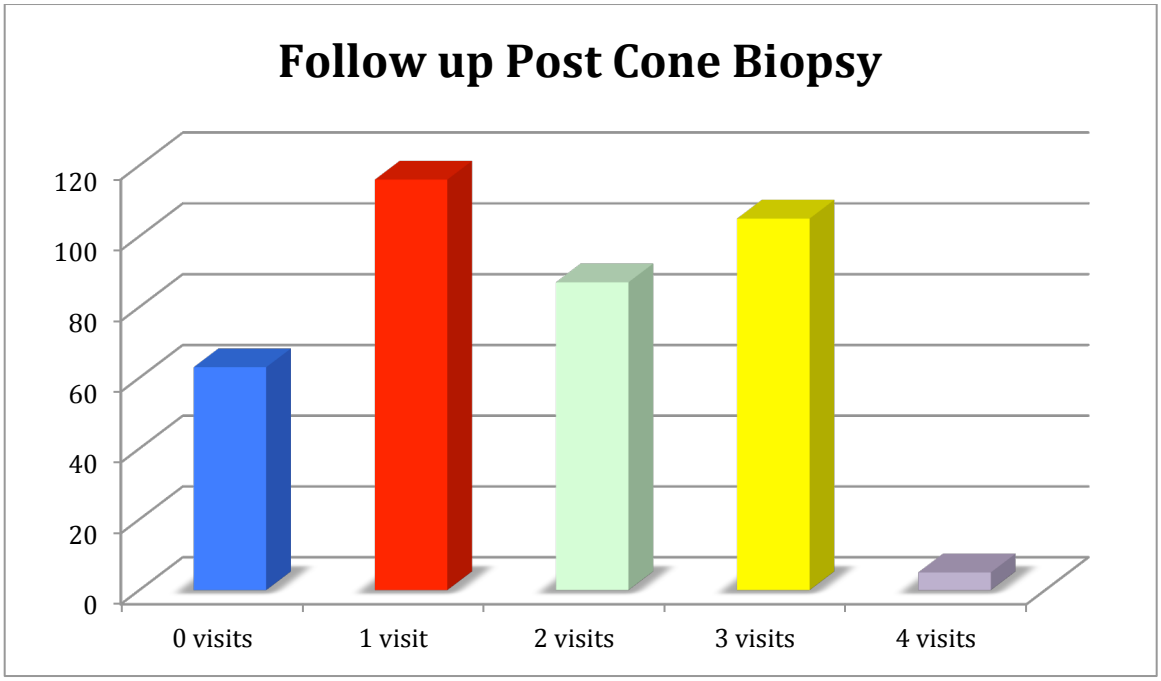


FIGURE 2.5 Number of visits Post Cone Biopsy

At the 4-6 month follow-up, data was available for 276 patients. Of these 84,4% (233/276) had a Pap smear or cervical biopsy performed. Hysterectomy was performed for 11,3% (31/276) of women :19 radical hysterectomies and 12 simple hysterectomies

(two of which were performed after a second cone biopsy). Repeat cone biopsy was required for 2,9% (8/276) of women and a LLETZ for 4 women. The results of the combined cytology and histology obtained at this visit were as follows: the majority of women 57,6% (159/276) had normal cytology and histology, but 13,4% (37/276) had LSIL and 17,4%(48/276) had HSIL. There were 7 patients with ASC-H and 6 patients with ASCUS. In addition there were 19 cancers detected – 18 of these women had cancer detected in the original cone biopsy and then underwent hysterectomy by the 4 month visit and one patient had a simple hysterectomy for persistent HSIL with cancer detected on the hysterectomy specimen.

At the 10-12 month follow up visit, data was available for 182 patients only: 47,7% of patients (166/348) either defaulted or there was no data. Of the 182 women seen, 88,5% (161/182) had a Pap smear/cervical biopsy or vault smear performed, 4,9% (9/182) required a repeat cone biopsy, 3,3% (6/182) a LLETZ, 2,2% (4/182) a simple hysterectomy and 0,5% (1/182) a radical hysterectomy. One patient had a colpectomy for high grade VAIN (vaginal intraepithelial neoplasia). When combining the results of the cytology and histology obtained at this visit, 64,3%(117/182) of women had normal cytology and histology, 17,0% (31/182) had HSIL, 13,7% (25/182) LSIL, 4 patients with ASCUS, and one with ASC-H. There were 2 cancers and 2 patients with VAIN 1-2.

Data was available for only147 patients at the more than 12 month follow-up visit : 57,4% (200/348) defaulted or had no data available. The majority of patients 87,8% (129/147) had a Pap smear or cervical biopsy performed, with 3,4% (5/147) requiring a

repeat cone biopsy and 4 patients a LLETZ. A further 5,4% (8/147) required a simple hysterectomy and 1 patient a vaginectomy. A review of cytology and histology at this visit showed that 70,1% (103/147) patients had normal results, 19,7% (29/147) had HSIL, 5,4% (8/147) LSIL and there were 4 patients with ASCUS. Three patients had VAIN and 1 specimen was suboptimal.

Cervical Cancer Management Post Cone Biopsy

Forty seven cancers were diagnosed or confirmed on cone biopsy during the study period, with 4 additional patients having cancer confirmed on a LLETZ followed by a cone biopsy showing no residual cancer. Ten patients were referred to radiation oncology and one demised shortly after diagnosis. Of the remainder, 11,8% (6/51) were followed up with colposcopy, pap smear and cervical biopsy and 56,9% (29/51) had a hysterectomy performed, which comprised 9 simple and 20 radical hysterectomies. The majority of these hysterectomies i.e. 93,1% (27/29) were performed by the 4 month visit and 2 were performed by the 10 month visit. Three patients required a repeat cone biopsy and one a LLETZ. One patient defaulted her surgical date and follow up appointments (and subsequently demised). This is shown graphically in Figure 2.6 below.

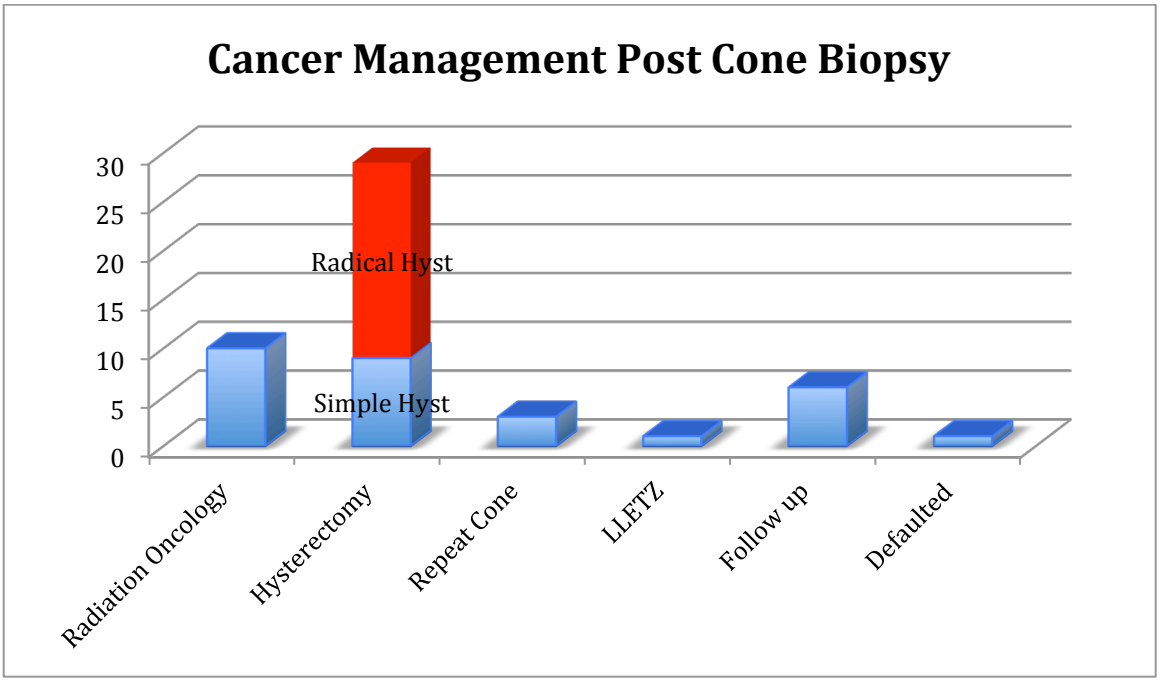


FIGURE 2.6 : Cervical Cancer management post Cone Biopsy

Twenty -nine hysterectomies were performed for cancer detected on cone biopsy. The histology of these specimens were as follows: 69,0% (20/29) were involved with cancer, 17,25% (5/29) were normal and 13,8% (4/29) had CIN 2/3. This is represented in Figure 2.7 below.

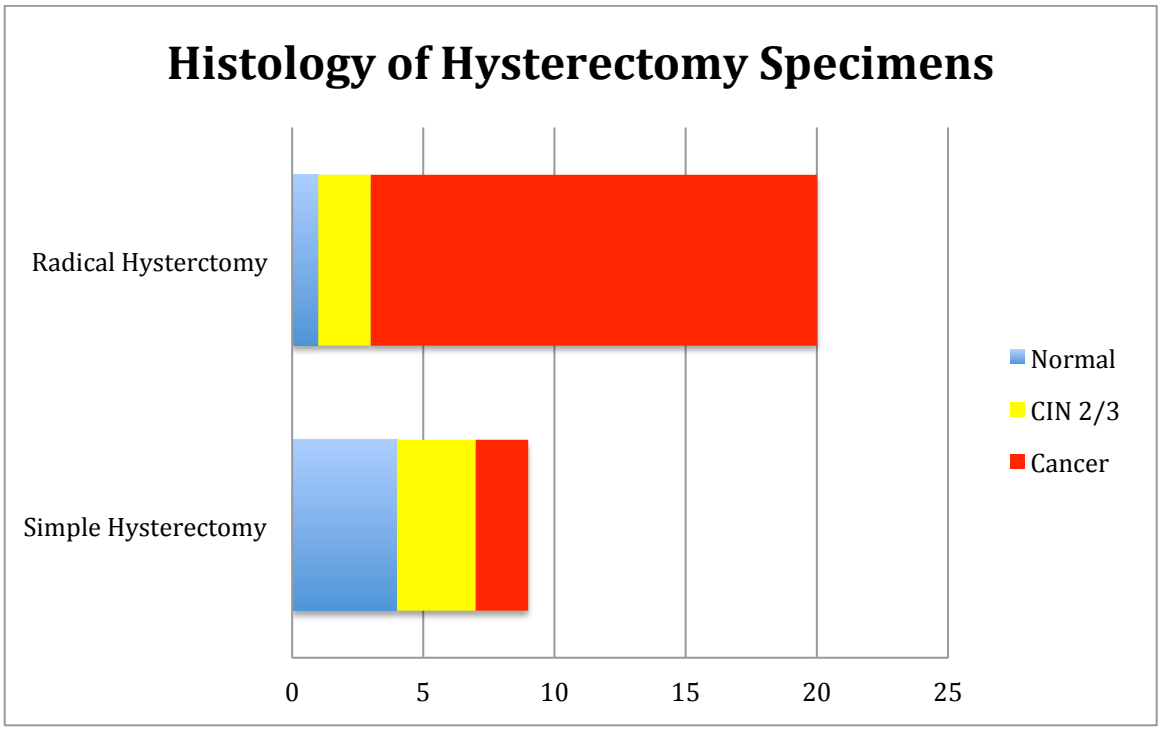


FIGURE 2.7: Histology of hysterectomy specimens

Predictors of persistent disease

Age, parity, HIV status, CD4 count, ARV status and cone biopsy margins were analysed as predictors of disease via logistic regression analysis. In the univariate analysis, persistent disease was proportionately more commonly diagnosed in women aged 40 – 49 years compared to younger or older women (Table 2) The relative risk was 1.3 [95% CI 0.9-1.9] but was not statistically significant.

AGE	**No. of women**	**%Persistent disease**	**Relative Risk**	**[95% CI]**	**P value**
<39	166	25.3	1.0	(ref)	-
40-49	123	34.2	1.3	0.9-1.9	0.101
>50	87	26.4	1.0	0.7-1.6	0.844

TABLE 2. Persistence of disease in different age categories

The mean parity was 2, with women in this category having a 1.2 fold increased risk of persistent disease as shown in Table 3 below, however this was not statistically significant.

PARITY	**No. of women**	**Persistent disease**	**Relative Risk**	**[95% CI]**	**p Value**
1	84	27.4	1.0	(ref)	-
2	114	32.5	1.2	[0.8-1.8]	0.446
3	81	25.9	0.9	[0.6-1.6]	0.833
≥ 4	96	28.5	1.0	[0.6-1.6]	0.964

TABLE 3 Persistence of disease related to parity

Persistent disease was found in similar proportions in HIV positive, HIV negative and women with unknown HIV status (Table 4).

HIV STATUS	**No. of women**	**%Persistent disease**	**Relative Risk**	**[95% CI]**	**P Value**
Negative	120	28.3	1.0	(ref)	-
Positive	213	29.1	1.0	[0.7-1.5]	0.881
Unknown	43	25.6	0.9	[0.5-1.6]	0.732

TABLE 4 Persistence of disease related to HIV status

The majority of women in the study who were HIV positive had CD4 counts between 250-349 (67/205), however disease was most commonly persistent in those women with CD4 between 350-499.There was a 1.2 fold increased risk of persistent disease in the group with CD4 counts between 350-499 [95% CI 0.6-2.2]. However, this was not statistically significant.

CD4	No. of women	Persistent disease(%)	Relative Risk	[95% CI]	P Value
0 – 199	46	28.3	1.0	(ref)	-
200-349	67	29.9	1.1	0.6 – 1.9	0.855
350 -499	45	33.3	1.2	0.6 – 2.2	0.601
>500	47	27.7	1.0	0.5 – 1.9	0.948

TABLE 5: Persistence of disease related to CD4 count

HIV status was positive in 213 women in the study. The majority of these women (151/213) were on ARV's. There was a 1.3 fold increased risk of persistent disease in the group of women in whom ARV status was unknown. Women on ARV's had a relative risk of 0.9 [95% CI 0.6-1.5]. However, none of these were statistically significant.

ARV's	No. of women	Persistent disease (%)	Relative Risk	[95% CI]	p Value
No	56	30.4	1.0	(ref)	-
Unknown	5	40.0	1.3	0.4-4.1	0.637
Yes	151	28.5	0.9	0.6-1.5	0.790

TABLE 6 : Persistence of Disease related to ARVs

In the univariate analysis , both endocervical and ectocervical margin involvement, were significantly associated with persistent disease if they were involved with CIN 2/3 and cancer, but not CIN1 (Table 7). When the endocervical margin was involved with cancer or CIN2/3 there was a 2.8 and 1.9 fold increased risk of persistent disease respectively, which was statistically significant.

ENDOCERVIX	**No. of Women**	**Persistent disease (%)**	**Relative Risk**	**[95% CI]**	**P Value**
Clear	201	20.4	1.0	(ref)	-
CIN 1	20	15.0	0.7	[0.3-2.2]	0.575
CIN 2/3	122	38.5	1.9	[1.3-2.7]	0.000
MI	23	56.5	2.8	[1.8-4.3]	0.000

TABLE 7 Endocervical Margin and persistence of disease

Similiarly, when analyzing the ectocervical margin, those with CIN 2/3 or MI margin involvement had a 2.1 and 2.2 fold increased risk of persistent disease respectively. Six patients were excluded from this analysis, 1 for AIS and the remainder for cautery artifact or unreported margins.

ECTOCERVIX	No of women	Persistent disease (%)	Relative Risk	[95% CI]	P Value
CIN 1	69	24.6	1.0	(ref)	-
Clear	212	21.7	0.9	[0.5 -1.4]	0.608
CIN 2/3	68	51.4	2.1	[1.3 -3.4]	0.002
MI	13	53,9	2.2	[1.1 -4.2]	0.019

TABLE 8 Ectocervical Margin and persistent disease

The multivariate analysis considered age, parity, HIV status and ecto- and endocervical margins as potential predictors of persistent disease. Women aged 40-49years had an increased risk of persistent disease (Adjusted RR 1.4 95% CI 1.01-2.0), which was noted to be statistically significant in the multivariate analysis (p=0.043). Endocervical margin involvement with CIN 2/3 or Cancer and ectocervical margin involvement with CIN2/3 were predictors of persistent disease and was statistically significant.

Variable	Level/ Category	N	%Persistent Disease	Crude RR [95%CI]	Crude P Value	Adjusted RR [95%CI]	Adjusted p Value
Age(in yrs)	<39	166	24.7	1.0 (ref)	-	1.0 (ref)	
	40-49	123	34.2	1.3[0.9-1.9]	0.101	1.4[1.01-2.0]	0.043
	>50	67	26.4	1.0 [0.7-1.6]	0.844	1.0[0.6-1.6]	
Parity	1	84	27.4	1.0 (ref)	-	1.0 (ref)	-
	2	114	32.5	1.2[0.8-1.8]	0.446	0.9[0.6-1.2]	0.403
	3	81	25.9	0.9[0.6-1.6]	0.833	0.7[0.5-1.1]	0.160
	≥4	96	27.1	1.0[0.6-1.6]	0.964	0.6[0.4-1.1]	0.099
HIV Status	Negative	120	28.3	1.0 (ref)	-	1.0 (ref)	-
	Positive	213	29.1	1.0[0.7-1.5]	0.881	1.1[0.8-1.5]	0.621
	Unknown	43	25.6	0.9[0.5-1.6]	0.732	1.1[0.6-1.8]	0.840
Endocervix Margin	Clear	201	20.4	1.0 (ref)	-	1.0 (ref)	-
	CIN 1	20	15.0	0.7[0.3-2.2]	0.575	0.8[0.2-3.2]	0.746
	CIN 2/3	122	38.5	1.9[1.3-2.7]	0.000	1.5[1.04-2.3]	0.031
	CA	23	56.5	2.8[1.8-4.3]	0.000	2.4[1.4-4.3]	0.003
Ectocervix Margin	CIN 1	69	24.6	1.0 (ref)	-	1.0(ref)	-
	Clear	212	21.7	0.9[0.5-1.4]	0.608	1.0[0.6-1.6]	0.890
	CIN 2/3	68	51.5	2.1[1.3-3.4]	0.004	1.8[1.1-3.0]	0.017
	CA	13	53.9	2.2[1.1-4.2]	0.019	1.3[0.6-2.7]	0.890

TABLE 9 Multivariate analysis of predictors of persistent disease

CHAPTER 4 : DISCUSSION AND CONCLUSION

Cervical cancer is the 4th commonest cancer worldwide and the 2nd commonest in South Africa. The marked discrepancy in low versus high resource settings is due to either nonexistent or poorly implemented cervical cancer screening programmes. Well organized screening programmes, as instituted by Nordic countries in the 1960's have been shown to effectively decrease morbidity and mortality associated with cervical cancer.

In South Africa, this has been compounded by the HIV epidemic with HIV positive women having a higher prevalence of HPV infection, persistent infection with HPV, infection with multiple types of HPV, higher prevalence of cervical cancer precursors and presentation at an earlier age with cervical cancer.

This highlights the need for effective screening and treatment programmes in South Africa. Our study analysed 376 women attending Groote Schuur Hospital Colposcopy Clinic, between 1st April 2010 and 31st October 2013, who required a cone biopsy with respect to demographics, cytology, histology and post cone compliance, management and follow up.

Demographics

The mean age of women requiring cone biopsy in our study was 42.3 yrs. Quantitative studies have shown that after two or more negative Pap smears, even screening once every 10 years yields a 64% reduction in the incidence of cervical cancer, assuming 100% compliance (Bulletin of the WHO, 1986). Well organized screening programs, with regular pap smears, have been shown to effectively decrease morbidity and

mortality associated with cervical cancer, unfortunately they involve substantial costs to provide for the infrastructure, human resources, equipment, and consumables required, which are often unaffordable in developing countries. The South African National cervical cancer screening programme offers 3 pap smears in a women's lifetime after the age of 30, each 10 years apart. Whilst far from ideal, the mean age of women in our study indicates that we are screening the correct age category (Sankaranarayanan, Budukh & Rajkumar 2001).

The majority of women in the study were parous, with a mean parity of 2. In the reproductive age group, just over a third of women (35,0%) were not using any form of contraception. Of those on contraception, a third (36,2%) were using long acting injectables and approximately 10% were sterilized. Only 12,5% of all women in the study used condoms and 17,2 % in the reproductive age group, placing most women at risk for HIV and HPV. The combined oral contraceptives were rarely used.

A large proportion of women in the study i.e. just under 60% were HIV positive. Numerous studies have previously been mentioned that detail the close association between HIV, HPV and cervical cancer supporting the high prevalence of HIV amongst the group of women in the study. However, given that the background HIV rate is lower i.e. 29% amongst antenatal patients in South Africa, this data must also be viewed in the context of HIV positive women more frequently accessing health services and receiving pap smears compared to HIV negative women. Of note was that the majority of HIV positive women, had low CD4 counts between 200-349 and that 70% were on ARV's.

Cytology and histology of Cone biopsy

The indications for cone biopsy reflected that one third were for disparity between cytology and colposcopy, a similar proportion for suspicion for microinvasion and a slightly higher percentage 38,6% for ULNS (upper limit of the lesion not seen). Only a minority, were for atypical glandular cells. Previous studies have shown ranges from 40-64% for ULNS, 16-26% for disparity and 1-12% for suspected microinvasion (Massad, Chronopoulos & Cejtin 1997; Spitzer et al 1998, El-Toukhy et al 2001). A study by El-Toukhy et al (2001) analysed 100 cold knife cone biopsies between 1987 and 1997, with a mean age of 41.8 years and parity 2. The main indications for cone biopsy were ULNS in 64% of patients, disparity in 26% and suspected microinvasion in 12%. CIN was histologically proven in 67% of cases, cancer in 7% and AIS in 3%. We can conclude, that while similar proportions of cone biopsies are performed for ULNS at other centers, a higher proportion are done for suspicion of microinvasion at GSH. This may reflect a higher burden of disease in our setting with 12,5% confirmed cancers on histology.

While histology of the cone biopsy was for high grade disease in most women i.e.65,4% (246/376), of note is that microinvasion was confirmed in 12,5% (47/376) of women and as predicted glandular lesions were infrequent. The negative cone rate was almost 6%, which is acceptable.

There has been conflicting data in the literature regarding the use of loop diathermy versus cold knife for cone biopsies, with regard to cautery artifact especially of the margins, fragmentation and interpretable results. Ioffe et al (1999) compared 24 cold knife cone biopsies with 76 LLETZ and found that all LLETZ had 1+ artifact and in 61% of cases it interfered with at least one aspect of evaluation but this was reduced if the LLETZ was received in a single specimen. Miroshnichenko et al (2009) found that loop electrosurgical excision was associated with an increased number of specimens that limited interpretability and an increased number of positive margins. In comparison another study by Huang & Hwang (1999) found smaller mean diameters in the base and depth of cone specimens and significantly shorter operating times with loop electrosurgical excision procedures compared to cold knife cone biopsies. In addition although thermal artifacts of the margin were found in 8.2% of LEEP specimens, there was no difficulty on histological interpretation.

In our study the pathologists were able to comment on margins in the majority (96%) of patients, with cautery artifact and lost epithelium in only a few patients. This demonstrates that loop electrosurgical excision is a viable option in our setting, where most cone biopsies are done on an outpatient basis, especially in light of limited theatre time, resources and compliance with follow up.

Unfortunately, there was a high rate of margin involvement, with only just under 60% of ectocervical margins and 54,6% of endocervical margins clear of disease. Where involved, the pathologists were able to comment on margins in the majority (96%) of patients, with cautery artifact and lost epithelium in only a few patients. When the

ectocervical margin was involved, this was due to CIN 1 in 18,8% (69/368) of cases, CIN 2/3 in 18,5%(68/368) of cases and microinvasive disease in 3,5%(13/368) of cases. In comparison the endocervical margin was involved with CIN2/3 in 33,2% of specimens, microinvasive disease in 6,3%(23/368) and CIN 1 in 5,4% (20/368) of specimens. This highlights the greater degree of disease involving the endocervical margin. A retrospective analysis by Mohamed-Noor, Quinn &Tan (1997) in Melbourne, Australia reviewed 699 cone biopsies between 1966 and 1992. Abnormal epithelium was completely excised in in 82% (572/699) cases and was incompletely excised in 18% (127/699). In those with complete excision, 96,7% were found to have been cured of disease at follow up or normal histology of hysterectomy specimens. The overall cure rate after incomplete excision was 77%, but was influenced by the site of incomplete excision. The cure rate for incomplete excision at the ectocervical margin was 86%, incomplete excision at the endocervical margin 68% and at both margins 40% demonstrating a risk of recurrence with incomplete excision particularly if the endocervical margin was involved.

Shaco- Levy at al (2014) reviewed 376 women that had cone biopsies performed between January 2001 and July 2011. Cone margin involvement was observed in 33% of women, with endocervical involvement in 22%, ectocervical in 8%, and both margins in 3% of women. They found that cone margin involvement had a statistically significant association with persistent/recurrent disease (focal: hazard ratio =17, $p<0.001$; extensive: hazard ratio=28, $p<0.001$), particularly when extensively involved.

Our study demonstrated a high rate of positive cone margin involvement, with 42,4% of ectocervical and 45,4% of endocervical involvement, and in particular endocervical

involvement with high grade and microinvasive disease. This is concerning when compared to international literature, which demonstrated significantly lower percentages of margin involvement but showed significant impact on persistent and recurrent cervical disease with positive margins. One possible cause for increased margin involvement, is larger lesions due to the generally unscreened population group that present to our colposcopy clinic.

Forty seven cases of microinvasion were detected directly via cone biopsy (with an additional 4 cases of microinvasion detected on previous LLETZ followed by a no residual cancer on cone biopsy). The majority of these 84,3% (43/51) were for squamous carcinoma which is the commonest histological type of cervical cancer and 9,8% (5/51) were adenocarcinoma. Moodley & Moodley (2009) conducted a retrospective study on 2930 women from 1995-2002 in the public and private sector in Durban, South Africa. They found squamous cell carcinoma to be the commonest type of cervical cancer in the public sector occurring in over 80% of patients, correlating with our findings. There was a larger percentage of adenocarcinoma in the private sector but this was not statistically significant and involved a small number of patients.

Of note, was that 56% (26/51) of cancers were stage 1A and 33% (17/51) were Stage 1B. Seven patients had Stage 2 disease and one patient had Stage 4 cancer. Encouragingly this reflects the detection of early stage cancer allowing for early treatment and improved outcome in screened populations. Hung et al (2014) considered both the survival and financial benefits of detection of invasive cervical cancer at earlier stages and estimated

the savings in life years and costs from early diagnosis of cervical cancer using an ex post approach. Their study reviewed 28 797 patients diagnosed with cervical cancer in the period 2002 and 2009 and found that invasive cervical cancer at stages 1-4 had an average expected years of life lost of 6.33years, 11.64 years, 12.65 years and 18.61 years respectively (Figure 3.1), while the lifetime costs paid by the NHI were $ 7020, $10 133, $11 120 and $10 015 US dollars respectively. The mean lifetime costs for managing cervical cancer were generally lower for stage 1 compared with stage 2, 3 and 4.

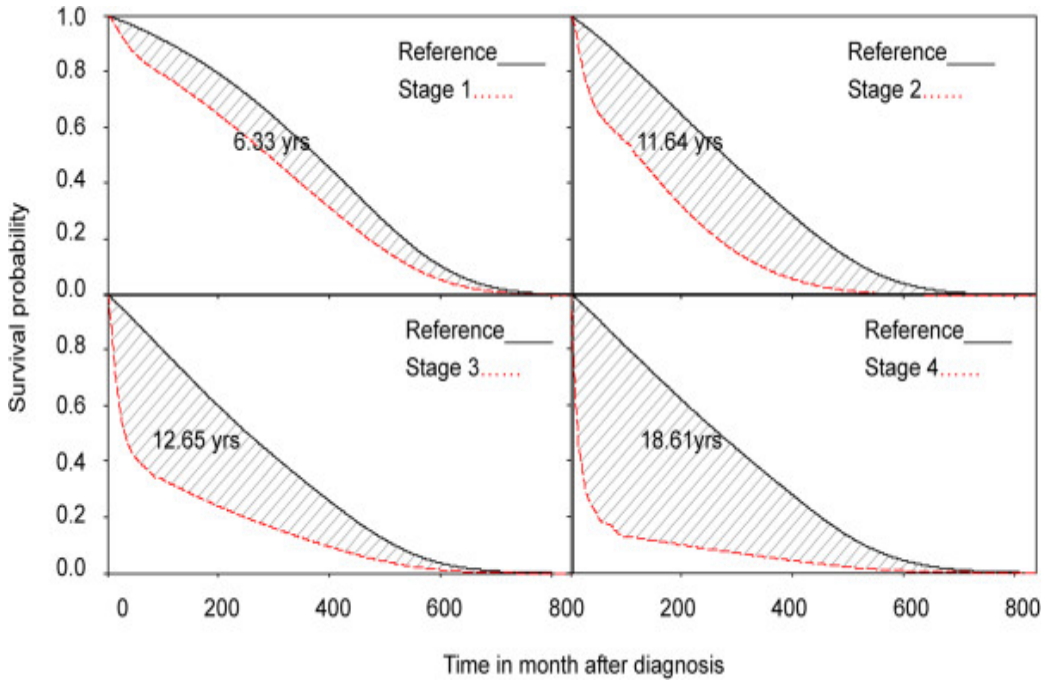


FIGURE 3.1 : Average expected years of life lost (EYLL) due to cervical cancer stratified by stages (The difference (shadowed area) of LE between the cohort of cervical cancer and age-, gender-matched reference population, which represents the average EYLL of developing a case of cervical cancer)

(Hung MC,Liu MT, Cheng YM &Wang JD 2014, *Estimation of savings of life years and cost from early detection of cervical cancer; a follow-up study using nationwide databases for the period 2002-2009,* BMC Cancer, vol 14, no 505)

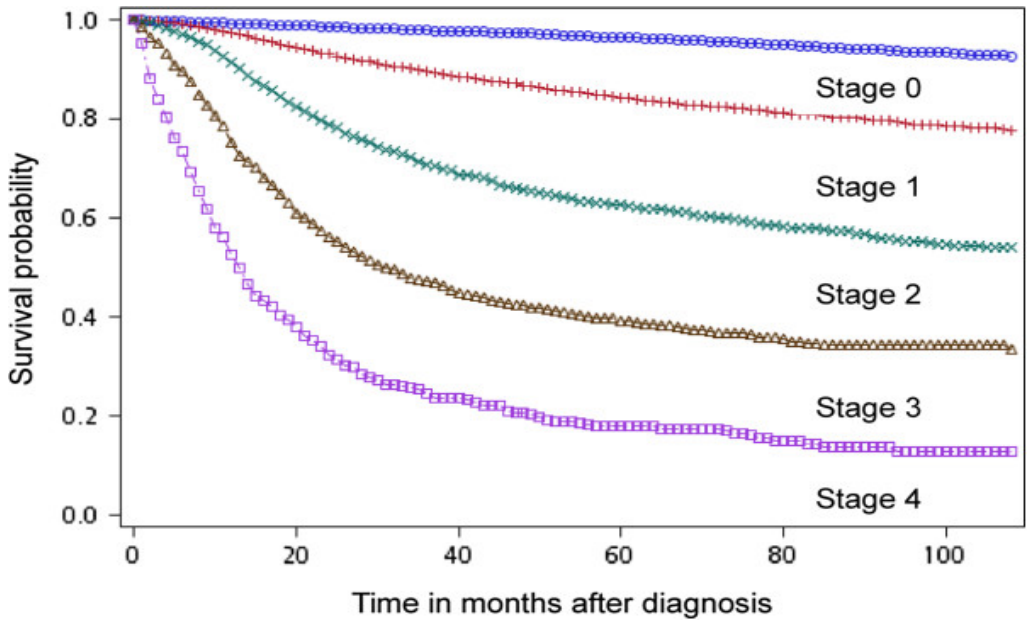


FIGURE 3.2: Survival probability of cervical cancer stratified by stages.

(Hung MC,Liu MT, Cheng YM &Wang JD 2014, *Estimation of savings of life years and cost from early detection of cervical cancer; a follow-up study using nationwide databases for the period 2002-2009,* BMC Cancer, vol 14, no 505)

Post Cone Management and Follow up

Unfortunately, follow up was relatively poor in this group of women with 17,3% (63/365) of women completely lost to follow up. Of the remainder only 31,8%(116/365) returned for 1 visit, 23,8%(87/365) for 2 visits, 28,8%(105/365) for 3 visits and only 5 patients had four visits.

Several studies have looked at predictors of cervical screening adherence, which can be extrapolated to post cone biopsy follow up. A systematic review by Limmer et al (2014) demonstrated several consistent variables associated with screening and follow up. A secure financial status, higher level of education, marriage, a high level of acculturation and good psychosocial health were positively associated with adherence among US adult

women. Similiarly, a recent Denmark study found that women with limited or no use of primary health care services, those with primary school education only, unmarried women and foreign nationals were poorly compliant (Kristensson et al 2014). Closer to home, a study by Batra, Kuhn & Denny (2010) et al looking at the utilization and outcomes of cervical cancer prevention services amongst HIV infected women in Cape Town found that only 13% of women were receiving at least one pap smear after HIV diagnosis. Most women referred for colposcopy tended to be HIV positive, more than 30 years of age and had high grade dysplasia (70%). HIV positive women treated with excision were significantly more likely than their HIV negative counterparts to undergo incomplete excision, experience persistent disease after treatment and lost to follow up. In contrast, a study involving a cohort of HIV positive US women found that better colposcopy compliance was associated with less education (OR 2.24, 95% CI 1.12-4.51), previous abnormal pap result, study site and higher stress. In this study 68% (222/378) of women were compliant with colposcopy referral (Massad et al 2012).

Interestingly, a study by Greenspan et al (2007) found that the type of excisional procedure was significant in predicting compliance. With 74.1% of cold knife cone biopsies compliant with follow up compared to 43.2% of LEEP patients. (adjusted RR 1.64 95% CI 1.3-1.87). Older patients were noted to be more compliant, in the univariate but not the multivariate analysis. Severity of disease, race and gravidity were not significant predictors. They concluded that given that LEEP is a less invasive in-office procedure, it may convey to patients that their condition is less severe.

At the 4-6 month follow up data was available for 276 patients with the majority 84,4% (233/276) requiring follow up with Pap smear or cervical biopsy. Of the 31 hysterectomies (19 radical and 12 simple hysterectomies) performed by this visit 27 were for cancer detected on cone biopsy and 4 were simple hysterectomies for persistent HSIL, one of which showed cancer. Repeat cone biopsy was required for 2,9%(8/275) of women and LLETZ for 4 patients. The combined cytology and histology at this visit showed that the majority of women 57,6% (159/276) were normal, 13,4%(37/276) had LSIL and 18,3%(48/276) had HSIL. Of the 31 hysterectomies performed, 7 were normal, 5 showed CIN 2/3, and 19 showed cancer. Thus, a significant proportion of women i.e. 24,3% (67/276) demonstrated persistent/residual disease post cone biopsy at this visit.

At the 10-12month follow up, data was unfortunately unavailable for 47,7% (166/348) of patients. The majority of women at this visit 88,5% (161/182) required follow up with Pap smear or cervical biopsy, 8,2%(15/182) patients required a repeat excisional procedure, 5 required a simple hysterectomy and 1 a radical hysterectomy. While 64,3% of women had normal cytology and histology, 19,2% (35/182) demonstrated persistent/recurrent disease with HSIL, cancer or VAIN present on cytology or histology.

Data was only available for 148 patients at the more than 12 month follow up with 87,8% requiring follow up via Pap smear or cervical biopsy , 6,08% (10/147) requiring a repeat excisional procedure, 9 patients a simple hysterectomy and 1 patient a vaginectomy. Again 20,4% (30/147) had persistent/recurrent disease with HSIL or VAIN on cytology or histology. (Compliance at this visit must be reviewed with caution as patients with a

normal 10-12month follow up visit seen after July 2013 would have a more than 12 month visit scheduled after the study period which may alter data).

A considerable loss to follow up was noted in our study, as highlighted above. Loss to follow up is a feature of colposcopy clinics globally, impacted upon by both patient and institutional factors, many of which have been discussed earlier. An important factor, is that follow up requires women without symptoms and a lack/poor understanding of the intention of screening to attend regular clinic visits, which sadly results in failure to do so. These are considered random events, with little effect on data interpretation and not resulting in systematic error.

Cervical Cancer Management Post Cone Biopsy

Fifty one cancers were detected during the study period with ten patients referred to radiation oncology and one patient having demised shortly after diagnosis. Just under 60% (29/51) of women had a hysterectomy performed, 20 radical and 9 simple hysterectomies. Almost 70% (20/29) of the hysterectomies performed showed residual cancer and 13,0%(4/49) demonstrated CIN 2/3. Only 17,3%(5/29) were normal. Sixteen of the nineteen radical hysterectomies and 2 of the 8 simple hysterectomies were involved with residual cancer.

Of the remainder of patients with cancer, 3 had repeat cone biopsies, 1 patient had a LLETZ and 11.8% (6/51) were followed up with colposcopy, pap smear and/or cervical biopsy (one of these patients was medically unfit for further oncological intervention). None of these patients demonstrated any residual cancer, however 5 of them had HSIL on

subsequent histology and cytology. These patients were aged between 25-39years, with one patient aged 47yrs and the majority (7/9) were stage 1Ai. The other two were stage 1Aii and 1Bi.

Cone biopsy with adequate margins and pelvic lymphadenectomy is now being considered as a treatment option for early stage cancer in young women requiring fertility sparing surgery. Lee et al (2014) performed a retrospective analysis of 169 patients who had Stage 1Ai cervical cancer after cone biopsy, between 1997 and 2007 in Seoul. During the study period, 18 patients had a cone biopsy as definitive treatment and 151 patients subsequently underwent simple or radical hysterectomy with lymphadenectomy. No parametrial involvement or lymph node metastasis was noted in any of the patients. Of the 62 patients who had negative resections, only one presented with residual tumour in subsequent surgery and only one recurrence of disease was identified in the total sample of 169 women with a median follow up time of 99 months.

The study data suggests that in our setting cone biopsy may be an appropriate treatment option for well selected young patients with early stage cervical cancer preferably stage 1Ai. However, given the persistence of high grade disease noted on follow up these patients require extensive counseling regarding the risk of recurrence and need for close monitoring, and compliance with follow up.

Predictors of Persistent disease

Vast arrays of studies have looked at predictors of persistent/residual disease post cone biopsy with conflicting results. Significant predictors that have been implicated in persistence have been increasing age(> 35years), parity and severe dysplasia. High risk HPV, positive cone margins and positive endocervical curettage have been shown to be statistically significant as predictors of residual/ recurrent disease. In our setting HR-HPV is unavailable and endocervical curettage is not used as part of standard protocol.

Age, parity, HIV status including CD4 and ARV status as well as cone biopsy margin involvement were included in the univariate analysis and age, parity and HIV status were analysed in the multivariate analysis. Persistence was defined as a LLETZ, Cone biopsy, hysterectomy or colpectomy required on follow up or CIN 2/3, Cancer or VAIN present on histology or cytology at follow up.

In the univariate analysis women aged 40-49 years had a slightly increased risk of persistent disease (34.2%) than younger or older women with a Relative Risk of 1.3 [95% CI 0.9-1.9; p value 0.101], but this was not statistically significant.
In addition, both ectocervical and endocervical margin involvement with CIN 2/3 and MI were associated with an increased risk of persistent disease. Where the endocervical margin was involved with CIN there was 38.5% persistent disease (RR1.9 95% CI 1.3-2.70) which was statistically significant p=0.000. There was a increased risk when the

endocervical margin was involved with microinvasive disease with 56.5% persistence (RR 2.8 95% CI 1.8-4.3).

When the ectocervical margin was involved with CIN 2/3, there was 51.4% persistence of disease (RR 2.1 95% CI 1.3-3.4: p value= 0.002). Involvement with microinvasion increased this persistence marginally to 53.9% (RR 2.2 95% CI 1.1-4; p value = 0.019).

Interestingly, whilst 56,7% (213/276) of women in the study were HIV positive, their positive status was not associated with an increased risk of persistent disease in the univariate analysis[RR 1.0 95% CI O.7-1.5]. HIV positive and negative women reflected the same risk of persistent disease, and there was a slightly decreased risk in those with unknown status (RR 0.9 95% CI 0,5-1,6]. Most HIV positive women had CD4 counts between 200 and 349, but only those with CD4 counts between 350-499 had a slightly increased risk of persistent disease, that was not statistically significant (RR 1.2 95%CI 0.5-1.9).Whilst one might intuitively expect HIV positive women, who present more frequently for colposcopy and demonstrate high grade lesions, to have greater persistence of disease this may be explained by the fact that 70% of HIV positive women in the study were on ARV's. In the univariate analysis, women on ARVS had a slightly decreased risk of persistence (RR 0.9 95%CI 0.65-1.5) and those whose ARV status was unknown a marginally increased risk (RR 1.3 95%CI 0.4-4.1). Ahdieh-Grant et al (2004) assessed the association between the use of Highly Active Antiretroviral Therapy (HAART/ARVS) and regression of squamous intraepithelial neoplasia in HIV infected women enrolled in the Women's Interagency HIV study, a large multicenter, prospective cohort study. Of the 2059 HIV infected participants, 312 HIV infected women had

normal cervical cytology at baseline and were subsequently diagnosed during 7years of follow-up with incident squamous intraepithelial neoplasia. Pap smears, CD4+ T cell counts and information regarding use of HAART was obtained every 6 months. Of the 312 women, 141 had lesions that regressed to normal cytology with a median time to regression of 2.7years. Overall, the incidence of regression increased over time after HAART was introduced. Before HAART was introduced, the rate of regression was 0,0% (95% CI 0,0%-2,4%). After HAART was introduced, the rate was 12,5% (95% CI 9.9% to 15.1%) and was related to post HAART CD4+T cell counts (p value=0.002). They noted that HAART was associated with an increased regression of SIL among HIV infected women, and among women who used HAART. However, the majority of cervical lesions among HIV infected women, even among individuals who used HAART, did not regress to normal.

Of note, is that we did not have access to viral loads in our study, which more recent literature has shown to be a more important predictor of outcome than CD4 counts.

In the multivariate analysis, age, parity, HIV status and margin involvement were analysed as predictors of persistent disease. Parity and HIV status were not associated with an increased risk of persistent disease. Women aged 40-49 years demonstrated an increased risk of persistent disease, which was statistically significant (Adjusted RR 1.4 :95% CI 1.01-2.0; p value =0.043). Endocervical margin involvement with CIN 2/3 (Adjusted RR1.5 95% CI 1.04-2.3: p value =0,031) and microinvasion (adjusted RR 2.4 95%CI 1.4-4.3) were significantly associated with an increased risk of persistent disease. This was statistically significant.

In the univariate analysis ectocervical involvement with CIN 2/3 and cancer were significantly associated with persistent disease, however in the multivariate analysis only ectocervical margin involvement with CIN 2/3 was associated with an increased risk of persistence (Adjusted RR 1.8 95%CI 1.1-3.0; p value =0.017).

CONCLUSIONS

In this study we conducted an audit of the cone biopsies performed at Groote Schuur Colposcopy clinic between 1st April 2010 and 31st October 2013 with respect to demographics, indications for cone biopsy, histology of the cone biopsy, post cone management, compliance and follow up.

Three hundred and seventy six cone biopsies were conducted during the study period, with a mean age of 42.3 years and mean parity of 2, supporting current South African National Screening Guidelines and reiterating that we are screening the correct age category of patients. Of concern was that almost 50% of women in this group were not using any form of contraception and only 11% were using condoms, placing them at risk of unwanted pregnancy, HIV and HPV. Almost 60% (213/376) of women in the study were HIV positive, with the majority having CD4 counts between 200 -349 and 70% of HIV positive women on ARV's. While this supports the association between HIV and high grade cervical dysplasia it may also demonstrate that HIV positive women have more access to healthcare, Pap smears and colposcopy referral than HIV negative women.

The indications for cone biopsies were appropriate, evidenced by the fact that high grade dysplasia was confirmed on almost two thirds of specimens and microinvasion on 12,5% (47/376) with a negative cone rate of 5,9%. Unfortunately, there was a significant

proportion of positive margin involvement particularly of the endocervical margin, contributing to persistent disease.

Fifty one cancers were detected during the study period, with 84% of these being early stage disease detection thus improving the survival and outcome for these patients as well as the financial burden associated with advanced stage disease. Twenty-nine hysterectomies were performed for cancer in the study with 9 patients followed up with repeat excisional procedure or with colposcopy, Pap smear or cervical biopsy. These were young patients with early stage disease that did not demonstrate any residual cancer but did have high-grade disease on follow up. The study data suggests cone biopsy may be an appropriate treatment option for well selected young patients with early stage cervical cancer preferably stage 1Ai in the reproductive age group. However counseling regarding the risk of recurrence/persistence and the need for close monitoring, and compliance with follow up is required. Given that this was a very small group of women, further studies are required to determine if this a feasible option in our setting.

Unfortunately, follow up was poor in this group of women and is an area that needs attention to decrease the burden of disease as well as disease progression. Counseling and education, improved psychosocial health, easier/local access to treatment sites, financial assistance as well as patient tracing and call back are some of the areas that must be reviewed to improve patient compliance and follow up.

Age, parity, HIV status, CD4 count, ARV status and cone margin involvement were analysed as predictors of persistence disease. In the multivariate analysis, age 40-49years, ectocervical margin involvement(with CIN 2/3) and endocervical margin involvement were all associated with an increased risk of persistent disease that was statistically significant. This subgroup of patients, should be closely monitored for persistent disease. Parity, HIV status and CD4 count were not associated with persistent disease. However HIV positive patients on ARVs demonstrated a slightly decreased risk of persistent disease in the univariate analysis, which may reflect disease regression for patients on ARVs. This is an area that needs further investigation and study.

In conclusion, the use of cone biopsy is a valid diagnostic and sometimes therapeutic procedure at Groote Schuur Hospital with significant detection of high grade disease and cervical cancer. Women aged 40-49years and positive cone margins are strong predictors of persistent disease. Improved compliance and a reduction in positive margins are two areas that need to be addressed to improve the current treatment programme. Use of cone biopsy for early stage cancer appears promising but needs further study.

REFERENCES:

Ahdieh-Grant, L., Li, R., Levine, AM., Massad, LS., Strickler, HD., Minkoff, H., Moxley, M., Palefsky, J, Sacks, H, Burk, RD, Gange, SJ. 2004. Highly active antiretroviral therapy and cervical squamous intraepithelial lesions in Human immunodeficiency virus-positive women. *Journal of the National Cancer Institute*. 96(14): 1070-1076.

Babkina, N, Heller, DS, Goldsmith, LT, Houck, KL. 2014. Cervical conization for cervical intraepithelial neoplasia (CIN) 2 and 3 in HIV-positive women: A Case-Control Study. *Journal of Lower Genital Tract Disease*. DOI: 10.1097/LGT.0000000000000058 [5th September 2014].

Batra, P, Kuhn, L, Denny,L. 2010. Utilisation and outcomes of cervical cancer prevention services among HIV-infected women in Cape Town. *South African Medical Journal*. 100(1): 39-44.

Bouchard-Fortier, G, Reade, CJ, Covens, A. 2014. Non-radical surgery for small early-stage cervical cancer. Is it time? *Gynaecology Oncology*. 132(3): 624-627.

Costales, A.B., Milbourne, A.M., Rhodes H.E., Munsell, M.F., Wallbillich, J.J., Brown, J, Frumovitz, M., Ramondetta, L.M., Schmeler, K.M. 2013. Risk of residual disease and invasive carcinoma in women treated for adenocarcinoma in situ of the cervix. *Gynecology Oncology*. 129(3): 513-516.

Cox, JT. 2006. The development of cervical cancer and its precursors: what is the role of human papillomavirus? *Current Opinion in Obstetrics and Gynecology*. 18(1): s5-s13.

Denny, L. 2012. Cervical cancer prevention: New opportunities for primary and secondary prevention in the 21st century. *International Journal of Gynecology and Obstetrics*. 119: S80-S84.

Denny, L., Boa, R., Williamson, AL., Allan, B., Hardie, D., Stan R., Myer, L. 2008. Human papilloma virus infection and cervical disease in human immunodeficiency virus-1 infected women. *Obstet Gynecol*. 111(6): 1380-1387.

Denny, L., Kuhn, L., De Souza, M., Pollack, AE., Dupree, W., Wright, TC Jr. 2005. Screen-and-treat approaches for cervical cancer prevention in low resource settings. A randomized controlled trial. *JAMA*. 294: 2173-2181.

Dolei, A., Curreli, S., Marongui P. et al.1999. Human immunodeficiency virus infection in-vitro activates naturally integrated human papillomavirus type 18 and induces synthesis of L1 capsid protein. *Journal Gen Virology*. 80: 2937-2944.

Ellerbrock, TV.,Chiasson, MA., Bush, TJ., Sun, XW., Sawo, D., Brudney, K., et al. 2000. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. *JAMA*. 283(8): 1031-1037.

Fanfani, F., Landoni, F., Gagliardi, ML., Fagotti, A., Preti, E., Moruzzi, MC., Monterossi, G., Scambia, G. 2014. Sexual and Reproductive Outcomes in early stage cervical cancer patients after excisional cone as a fertility- sparing surgery: an Italian Experience. *Journal of Reproduction & Infertility*. 15(1): 29-34.

Firnhaber, C.S., Michelow, P. 2009. Cervical cancer and the human immunodeficiency virus : a review. *Southern African journal of HIV Medicine*. 10.2: 23

Goldie, S.J., Kohli, M., Grima, D., Weinstein, M.C., Wright, T.C., Bosch, F.X., Franco,E. 2004. Projected benefits of a human papillomavirus 16/18 vaccine. *Journal of the National Cancer Institute*. 96(8): 604-615.

Greenspan, D.L., Faubion, M., Coonrod, D.V., Hart, K.W., Mathieson, K. 2007. Compliance after loop electrosurgical excision procedure or cold knife cone biopsy. *Obstetrics and Gynecology*. 110 (3): 675-680.

Hakama, M., Louhivuori, K. 1988. A screening programme for cervical cancer that Worked. *Cancer Survey*. 7(3): 403-416

Harris, T.G., Burk, R.D., Palefsky, J.M., Massad, L.S., Bang, J.Y., Anastos, K. et al 2005. Incidence of cervical squamous intraepithelial lesions associated with HIV serostatus, CD4 cell counts, and human papillomavirus test results. *JAMA*. 293(12): 1471-1476.

Huang, L.W., Hwang, J.L. 1999. A comparison between loop electrosurgical excision procedure and cold knife conization for treatment of cervical dysplasia: residual disease in a subsequent hysterectomy specimen. *Gynaecology Oncology*. 73(1): 12-15.

Hung, M.C., Liu, M.T., Cheng Y.M., Wang, J.D. 2014. Estimation of savings of life years and cost from early detection of cervical cancer: a follow-up study using nationwide databases for the period 2002-2009. *BMC Cancer*. 14:505. DOI:10.1186/1471-2407-14-505

Ioffe, O.B., Brooks, S.E., De Rezende, R.B., Silverberg, S.G. 1999. Artifacts in cervical LLETZ specimens : correlation with follow-up. *International Journal of Gynecology Pathology*. 18(2): 115-121.

International Agency for Research on Cancer, World Health Organization. 2012. *GLOBOCAN 2012*. Available from: <http://globocan.iarc.fr/.

Kobayashi, A., Greenblatt, R. & Anastos, K. et al .2004. 'Functional Attributes of mucosal immunity in cervical neoplasia andeffects of HIV infection. *Cancer Research*. 64:6766-6774

Kristensson, J.H., Sander, B.B., von Euler-Chelpin, M., Lynge, E. Predictors of non-participation in cervical screening in Denmark. *Cancer Epidemiology*. 38(2):174-180.

Laara, E., Day, N.E., Hakama ,M. 1987. Trends in mortality from cervical cancer in Nordic countries: association with organized screening programmes. *Lancet*. 1(8544):1247-1249.

Lee, J.Y., Kim, H.S., Kim, K., Chung, H.H., Kim J.W., Park N.H., Song Y.S. 2014. Safety of less aggressive surgery for stage IA1 squamous cell carcinoma of the cervix. J*ournal of Obstetric and Gynaecology Research*. 40(5):1382-1388.

Leguevaque, P., Motton, S., Decharme, A., Soulé-Tholy, M., Escourrou, G., Hoff, J. 2010. Predictors of recurrence in high-grade cervical lesions and a plan of management. *European Journal of Surgical Oncology*. 36(11): 1073-1079.

Limmer, K., Lo-Biondo-Wood, G., Dains, J. 2014. Predictors of cervical cancer screening adherence in the United States: A systematic review. *Journal of the Advanced Practitioner in Oncology*. 5(1):31-41.

Lomalisa, P., Smith, T., Guidozzi, F. 2000. Human immunodeficiency virus infection and invasive cervical cancer in South Africa. *Gynecology Oncology*. 77(3): 460-463.

Lubrano, A., Medina, N., Benito, V., Arencibia, O., Falcón, J.M., Leon, L., Molina, J., Falcón, O. 2012. Follow-up after LLETZ: a study of 682 cases of CIN 2-CIN 3 in a single institution. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 161(1): 71-74.

Massad, S., Ahdieh, L., Benning, L., Minkoff , H., Greenblatt, R.M., Watts, H., Miotti, P., Anastos, K., Moxley, M., Muderspach, L.I., Melnick, S. 2001. Evolution of cervical abnormalities among women with HIV-1: evidence from surveillance cytology in the women's interagency HIV study. *Journal of Acquired Immune Deficiency Syndrome*. 27(5): 432-442.

Massad, L.S., Chronopoulos, F.T. & Cejtin, H.E. 1997. Correlating cone biopsy histology with operative indications. *Gynecology Oncology*. 65(2): 286-90.

Massad, L.S., Fazzari, M.J., Anastos, K., Klein, R.S., Minkoff, H., Jamieson, D.J., Duerr, A., Celentano, D. et al . 2007. Outcomes after treatment of cervical intraepithelial neoplasia among women with HIV. *Journal of Lower Genital Tract Disease*. 11(2):90-97.

Massad, L.S., Weber, K.M., Wilson, T.E., Goderre, J.L., Hessol, N.A., Henry, D., Colie,C., Strickler, H.D. et al . 2012. Correlating knowledge of cervical cancer prevention and Human papillomavirus with compliance after colposcopy referral. *Journal of Lower genital Tract Disease*. 16(2):98-105.

McDonald, A.C., Tergas, A.I., Kuhn, L., Denny, L., Wright, T.C. Jr .2014. Distribution of Human papillomavirus genotypes among HIV-Positive and HIV-Negative women in Cape Town, South Africa. *Front Oncology*. 14(4):48.

Miroshnichenko, C.G., Parva, M., Holtz, D.O., Klemens, J.A., Dunton, C.J. 2009. Interpretability of excisional biopsies of the cerix: cone biopsy and loop excision. *Journal Lower Genital Tract Disease*. 13(1):10-12.

MMWR Recomm Report. 1992. *Revised Classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults*. 41(RR-17): 1-9.

Mohamed-Noor, K., Quinn, M.A., Tan, J. 1997. Outcomes after cervical cold knife conization with complete and incomplete excision of abnormal epithelium: a review of 699 cases. *Gynecology Oncology*. 67(1) 34-38.

Moodley, M., Moodley, K. 2009. Trends of histopathology of cervical cancer among women in Durban, South Africa. *European Journal of Gynaeoclogical Oncology*. 30(2):145-150.

Moodley, M., Moodley, J., Kleinschmidt, I. 2001. Invasive cervical cancer and human immunodeficiency virus (HIV) infection: a South African perspective. *International Journal of Gynecological Cancer*. 11(3):194-197.

Moore, B.C., Higgins, R.V., Laurent, S.L., Marroum, M.C., Bellitt, P. 1995. Predictive factors from cold knife conization for residual intraepithelial neoplasia in subsequent hysterectomy. *American Journal of Obstetrics & Gynaecology*. 173(2): 366-368.

Mougin, C., Dalstein, V., Prétet, J.L., Gay, C., Schaal, J.P., Reithmuller, D. 2001. Epidemiology of cervical papillomavirus infections. Recent knowledge. *Presse Med*. 30(20):1017-1023.

Munoz, N., Bosch, F.X., de Sanjose, S., Herrero, R., Castellsague, X. & Shah, K.V. et al. 2003. Epidemiologic Classification of Human papillomavirus types associated with cervical cancer. *New England Journal of Medicine*. 348(6): 518-527.

National Cancer Registry Report. 2007. Available from: <http://www.nioh.ac.za>. [5 April 2014].

Ramchandani, S.M., Houck, K.L., Hernandez, E., Gaughan, J.P. 2007. Predicting persistent/recurrent disease in the cervix after excisional biopsy. *MedGenMed*. 9(2):24.

Sadler, L., Saftlas, A., Wang, W., Exeter, M., Whittaker, J., McCowan, L. 2004. Treatment for cervical intraepithelial neoplasia and risk of preterm delivery. *JAMA* . 291(17): 2100-2106.

Saeaib, N., Boonyapipat, S., Tungsinmunkong, K., Liabsuetrakul,T. 2009. Predictors of the residual disease of high-grade lesions and microinvasive squamous cell carcinoma of the cervix following conization. *Journal of Medical Association of Thailand*. 92(11):1406-1412.

Sankaranarayanan, R., Budukh, A. & Rajkumar, R. 2001. Effective screening programmes for cervical cancer in low-and middle- income developing countries. *Bulletin of the World Health Organization*. 79(10).

Sankaranarayanan, R., Esmy, P., Rajkumar, R., Muwonge, R., Swaminathan, R., Shanthakumari, S., Fayette, J.M., Cherian, J. 2007. Effect of visual screening on cervical cancer incidence and mortality in Tamil Nadu, India: a cluster-randomised trial. *Lancet*. 370:398-406.

Sankaranarayanan, R., Nene, S., Shastri, K. 2009. HPV screening for cervical cancer in rural India. *New England Journal of Medicine*. 360(14):1385-1394.

Shaco-Levy, R., Eger, G., Dreiher, J., Benharroch, D., Meirovitz, M. 2014. Positive margin status in uterine cervix cone specimens is associated with persistent/recurrent high grade dysplasia. *International Journal of Gynecological Pathology*. 33(1): 83-88.

Singh, D.K., Anastos, K., Hoover, D.R., Burk, R.D., Shi, Q., Nqendahayo, L., Mutimura, E., Cajigas, A. et al. 2009. Human papillomavirus infection and cervical cytology in HIV-infected and HIV-uninfected Rwandan women. *J Infect Dis*. 199(12):1851-61.

South African National Department of Health. 2011. *The National Antenatal Sentinel HIV and Syphilis Prevalence Survey, South Africa.*

South African National Department of Health. 2003. *National Guidelines for Cervical Cancer Screening Programme 2003*. Available from: <http://www.doh.org>.

Sozen, H., Namazov, A., Cakir, S., Akdemir, Y., Vatansever, D., Karateke, A. 2014. Pregnancy outcomes after cold knife conization related to excised cone dimensions. A retrospective cohort study. *Journal of Reproductive Medicine*. 59(1-2):81-86.

Spitzer, M., Chemys, A.E., Shifrin, A., Ryskin, M. 1998. Indications for cone biopsy: pathologic correlation. *American Journal of Obstetrics & Gynaecology*. 178(1):74-79.

Stanley, M. 2006. Immune responses to Human papillomavirus. *Vaccine*. 24(1): S16-S22.

Statistics South Africa. 2013. *Mid-year population estimates 2013*. Available from: <www.statssa.gov.za> .[6th April 2014].

Tillmanns, T.D., Falkner, C.A , Engle, D.B., Wan, J.Y., Mannel, R.S., Walker, J.L., Johnson, G.A., Mcmeekin, D.S. et al. 2006. Preoperative predictors of positive margins after loop electrosurgical excisional procedure- Cone. *Gynecology Oncology*. 100(2): 379-384.

El-Toukhy, T.A., Mahadevan, S. & Davies, A.E. 2001. Cold knife cone biopsy- a valid toll and treatment option for lesions of the cervix. *Journal Of Obstetrics and Gynaecology*. 21(2):175-178.

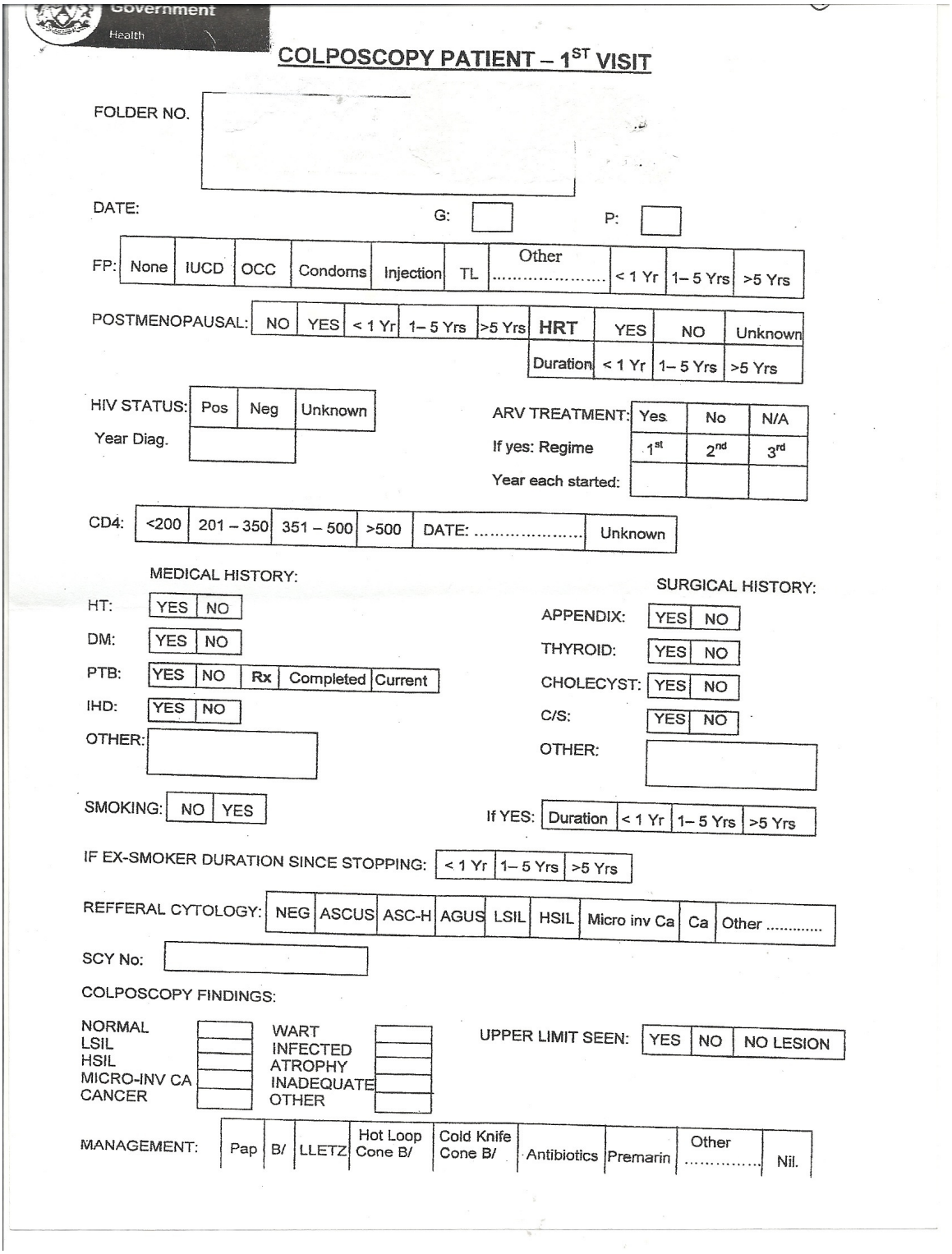
Vernon, S., Hart, C., Reeves, W. & Icenogle, J. 1993 .The HIV-1 Tat protein enhances E2-dependant human papillomavirus 16 transcription. *Virus Res*. 27:133-145.

Walboomers, J.M., Jacobs, M.V., Manos, M.M., Bosch, F.X., Kummer, J.A., Shah, K.V., Snijders, P.J., Peto, J. et al. 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *Journal Pathology*. 189:12-19.

Wright, T.C., Kurman, R.J. & Ferenczy. 2002. Precancerous lesions of the cervix. In *Blausteins Pathology of the Female genital Tract*. R.J. Kurman, Ed. 5th edn. New York: Springer-Verlag. 22-34.

APPENDICES :

APPENDIX A



Government
Health

COLPOSCOPY PATIENT – 1ST VISIT

FOLDER NO.

DATE: G: P:

FP: None | IUCD | OCC | Condoms | Injection | TL | Other ..... | < 1 Yr | 1– 5 Yrs | >5 Yrs

POSTMENOPAUSAL: NO | YES | < 1 Yr | 1– 5 Yrs | >5 Yrs | HRT | YES | NO | Unknown
Duration | < 1 Yr | 1– 5 Yrs | >5 Yrs

HIV STATUS: Pos | Neg | Unknown
Year Diag.

ARV TREATMENT: Yes | No | N/A
If yes: Regime | 1st | 2nd | 3rd
Year each started:

CD4: <200 | 201 – 350 | 351 – 500 | >500 | DATE: ..... | Unknown

MEDICAL HISTORY:

HT: YES | NO
DM: YES | NO
PTB: YES | NO | Rx | Completed | Current
IHD: YES | NO
OTHER:

SURGICAL HISTORY:

APPENDIX: YES | NO
THYROID: YES | NO
CHOLECYST: YES | NO
C/S: YES | NO
OTHER:

SMOKING: NO | YES

If YES: Duration | < 1 Yr | 1– 5 Yrs | >5 Yrs

IF EX-SMOKER DURATION SINCE STOPPING: < 1 Yr | 1– 5 Yrs | >5 Yrs

REFFERAL CYTOLOGY: NEG | ASCUS | ASC-H | AGUS | LSIL | HSIL | Micro inv Ca | Ca | Other .....

SCY No:

COLPOSCOPY FINDINGS:

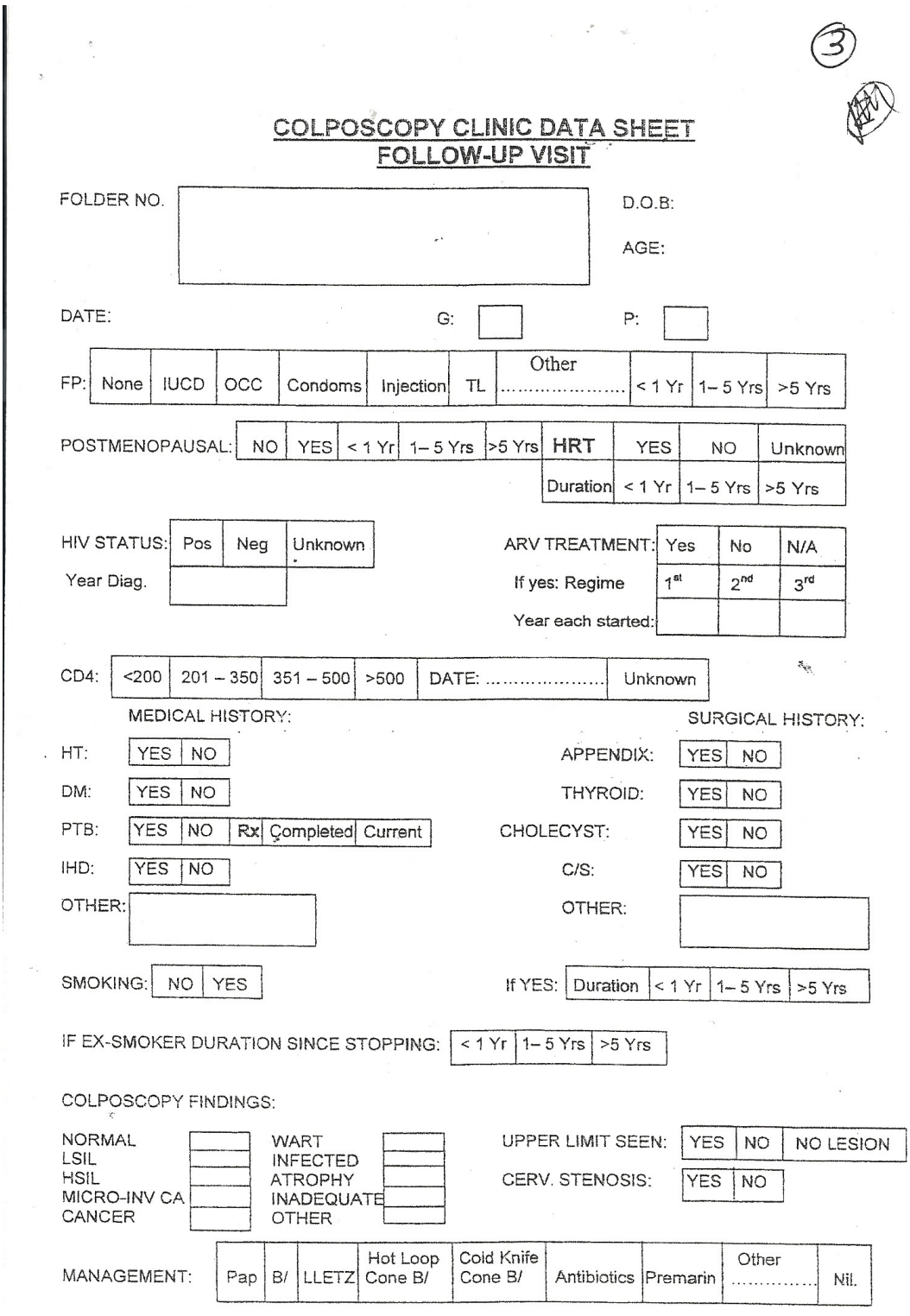
NORMAL
LSIL
HSIL
MICRO-INV CA
CANCER

WART
INFECTED
ATROPHY
INADEQUATE
OTHER

UPPER LIMIT SEEN: YES | NO | NO LESION

MANAGEMENT: Pap | B/ | LLETZ | Hot Loop Cone B/ | Cold Knife Cone B/ | Antibiotics | Premarin | Other ..... | Nil.

APPENDIX B



COLPOSCOPY CLINIC DATA SHEET
FOLLOW-UP VISIT

FOLDER NO. | D.O.B: | AGE:

DATE: | G: | P:

FP:	None	IUCD	OCC	Condoms	Injection	TL	Other	< 1 Yr	1– 5 Yrs	>5 Yrs

POSTMENOPAUSAL:	NO	YES	< 1 Yr	1– 5 Yrs	>5 Yrs	HRT	YES	NO	Unknown
						Duration	< 1 Yr	1– 5 Yrs	>5 Yrs

HIV STATUS:	Pos	Neg	Unknown
Year Diag.			

ARV TREATMENT:	Yes	No	N/A
If yes: Regime	1st	2nd	3rd
Year each started:			

CD4:	<200	201 – 350	351 – 500	>500	DATE:	Unknown

MEDICAL HISTORY:

HT:	YES	NO
DM:	YES	NO
PTB:	YES	NO	Rx	Completed	Current
IHD:	YES	NO
OTHER:		

SURGICAL HISTORY:

APPENDIX:	YES	NO
THYROID:	YES	NO
CHOLECYST:	YES	NO
C/S:	YES	NO
OTHER:		

SMOKING:	NO	YES

If YES: Duration | < 1 Yr | 1– 5 Yrs | >5 Yrs

IF EX-SMOKER DURATION SINCE STOPPING: | < 1 Yr | 1– 5 Yrs | >5 Yrs

COLPOSCOPY FINDINGS:

NORMAL		WART	
LSIL		INFECTED	
HSIL		ATROPHY	
MICRO-INV CA		INADEQUATE	
CANCER		OTHER	

UPPER LIMIT SEEN:	YES	NO	NO LESION
CERV. STENOSIS:	YES	NO	

MANAGEMENT:	Pap	B/	LLETZ	Hot Loop Cone B/	Cold Knife Cone B/	Antibiotics	Premarin	Other	Nil.

APPENDIX C

Western Cape Government
Health

COLPOSCOPY CLINIC DATA SHEET
1ST POST LLETZ / CONE VISIT

FOLDER NO. | D.O.B: | AGE:

DATE: | G: | P:

FP:	None	IUCD	OCC	Condoms	Injection	TL	Other	< 1 Yr	1– 5 Yrs	>5 Yrs

POSTMENOPAUSAL:	NO	YES	< 1 Yr	1– 5 Yrs	>5 Yrs	HRT	YES	NO	Unknown
						Duration	< 1 Yr	1– 5 Yrs	>5 Yrs

HIV STATUS:	Pos	Neg	Unknown
Year Diag.			

ARV TREATMENT:	Yes	No	N/A
If yes: Regime	1st	2nd	3rd
Year each Started:			

CD4:	<200	201 – 350	351 – 500	>500	DATE:	Unknown

MEDICAL HISTORY:

HT:	YES	NO
DM:	YES	NO
PTB:	YES	NO	Rx	Completed	Current
IHD:	YES	NO
OTHER:		

SURGICAL HISTORY:

APPENDIX:	YES	NO
THYROID:	YES	NO
CHOLECYST:	YES	NO
C/S:	YES	NO
OTHER:		

SMOKING:	NO	YES

If YES: | Duration | < 1 Yr | 1– 5 Yrs | >5 Yrs |

IF EX-SMOKER DURATION SINCE STOPPING: | < 1 Yr | 1– 5 Yrs | >5 Yrs |

LLETZ COMPLICATION:	YES	NO

IF YES: Bleeding: | YES | NO | Infection: | YES | NO |

REQUIRED RX:	YES	NO

ADMITTED TO HOSPITAL:	YES	NO

OTHER:

COLPOSCOPY FINDINGS:

NORMAL		WART	
LSIL		INFECTED	
HSIL		ATROPHY	
MICRO-INV CA		INADEQUATE	
CANCER		OTHER	

UPPER LIMIT SEEN:	YES	NO	NO LESION
CERV. STENOSIS:	YES	NO	

MANAGEMENT:	Pap	B/	LLETZ	Hot Loop Cone B/	Cold Knife Cone B/	Antibiotics	Premarin	Other	Nil.

APPENDIX D

RESULTS

FOLDER NO. _____ D.O.B

AGE

HISTOLOGY

SCA _____

NORMAL ☐

HPV ONLY ☐

CIN 1 ☐

CIN 2 , 3 ☐

SUSP.OF MICRO-INVASION ☐

MICROINVASION ☐

CANCER ☐

INADEQUATE ☐

OTHER ☐

CYTOLOGY

SCY _____

NORMAL ☐

ASC-H ☐

ASCUS ☐

AGUS ☐

LSIL ☐

HSIL ☐

SUSP. OF MICRO INVASION ☐

CANCER ☐

INADEQUATE ☐

CONE / LLETZ / EXCISION MARGINS

Clear ☐

Involved - Ecto cx line ☐

- Endo cx lin ☐

- Both ☐

If cancer: Adeno carcinoma ☐

Squamous cell carcinoma ☐

Adeno – squamous carcinoma ☐

VULVAL BIOPSY RESULTS

VIN 1-2 ☐

VIN 3 ☐

LSetA ☐

CANCER ☐

ECZEMA ☐

PSORIASIS ☐

LICHEN SIMPLEX ☐

FUNGAL INFECT. ☐

HERPES ☐

HPV ☐

OTHER ☐

HYSTERECTOMY YES | NO | NA

IF YES: Vaginal YES | NO

Abdominal YES | NO

Laparoscopic YES | NO

APPENDIX E

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492 • **Facsimile** [021] 406 6411
Email: Sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

10 July 2014

HREC/REF: 485/2014

Prof L Denny
Obstetrics & Gynaecology
H45 OMB

Dear Prof Denny

Project Title: AUDIT OF WOMEN MANAGED WITH CONE BIOPSY AT GROOTE SCHUUR HOSPITAL FROM 1ST APRIL 2010 TO 31ST DECEMBER 2013 (Masters Candidate - K Kadwa)

Thank you submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year until the 30 July 2015.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

We acknowledge that the following student/s:- Khatija Kadwa is also involved in this project.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

Signed

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

Hrec/ref:485/2014